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SYSTEMATIC REVIEW

Localised insulin administration for wound healing in non-diabetic adults: A systematic review and meta-analysis of randomised controlled trials

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Abstract

Insulin has the potential to restore damaged skin and due to its affordability and global availability, it is an agent of interest when it comes to pioneering new remedies to accelerate wound healing. The aim of this study was to explore the efficacy and safety of localised insulin administration on wound healing in non-diabetic adults. Studies were systematically searched, using the electronic databases Embase, Ovid MEDLINE and PubMed, screened, and extracted by two independent reviewers. A total of seven randomised controlled trials that met the inclusion criteria were analysed. Risk of bias was assessed using the Revised Cochrane Risk-of-Bias Tool for Randomised Trials and a meta-analysis was performed. The primary outcome, which explored rate of wound healing (mm^2/day), concluded that there was an overall significant mean improvement in the insulin treated group (IV = 11.84; 95% CI: 0.64–23.04; $p = 0.04$; $I^2 = 97\%$) compared to the control group. Secondary outcomes concluded that there is no statistical difference between the healing time (days) of the wound (IV = −5.40; 95% CI: −11.28 to 0.48; $p = 0.07$; $I^2 = 89\%$); there is a significant reduction in wound area in the insulin group; no adverse events were noted with the administration of localised insulin; quality of life improves drastically as the wound heals, irrespective of insulin. We conclude that although the study showed an improved wound healing rate, other parameters were not statistically significant. Therefore, larger prospective studies are warranted to fully explore the effects of insulin on different wounds, where an appropriate insulin regime can be developed for clinical practice.

KEYWORDS
insulin, non-diabetic, RCT, wound healing

Abbreviations: DLQI, Dermatology Life Quality Index; EGF, epidermal growth factor; FOXO, Fork head box protein O1; IGF-1, insulin-like growth factor 1; IL, interleukin; MEDLINE, Medical Literature Analysis and Retrieval System Online; NFkβ, nuclear factor kappa beta; PDGF, platelet-derived growth factor; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; QoL, quality of life; RCT, randomised controlled trials; RoB 2, Revised Cochrane Risk-of-Bias Tool for Randomised Trials; TGF-β, transforming growth factor beta; TNF-α, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor.

1 | INTRODUCTION

A wound is an injury to the skin that causes disruption to the continuity of tissue structure.[1] Every year, wounds affect approximately 2.2 million

individuals in the United Kingdom alone, where more than £5.3 billion is spent annually on treatment and management of associated complications[2]; this highlight how wounds can impose a significant burden not only to individuals, but to the healthcare system as a whole.

Wound healing is a complex biological process that involves clot formation, inflammation, granulation tissue development and remodelling.[3] This can be influenced by various agents such as insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF).[4] A variety of therapeutic methods are available to accelerate wound healing including skin grafts, hydrocolloid dressings, hyperbaric oxygen therapy, skin substitutes and negative pressure wound healing.[5,6] However, these can have associated complications and may not be feasible for certain patients due to wound-type, preference or expense. In the early stages of healing, the crucial process of re-epithelialisation takes place—this involves proliferation, migration and differentiation of keratinocytes from the wound margins.[3,7] Research suggests that insulin has the potential to enhance many of these processes, as well as increase blood flow and promote granulation tissue regeneration that would contribute to wound healing.[8–10]

Insulin is a well-known peptide hormone and growth factor that has the potential to restore damaged skin.[10] It has the ability to reduce inflammation by changing the expression of pro- and anti-inflammatory cytokines in the body.[11] This includes the activation of anti-inflammatory cytokines, including interleukin (IL)-10, IL-4 and VEGF, which inhibits cell apoptosis whilst promoting cell proliferation.[11,12] It also suppresses the protein transcription factor nuclear factor kappa beta (NFkβ) P50/P65, which decreases the expression of pro-inflammatory markers IL-6, IL-12 and tumour necrosis factor alpha (TNF-α).[11] This in turn accelerates regeneration and healing. Research show that insulin can also impact glucose metabolism, protein biosynthesis and lipid biosynthesis, which can promote wound healing.[11]

Due to its affordability and global availability,[13] insulin is an agent of interest when it comes to pioneering new remedies to accelerate wound healing. Systemic insulin treatment has presented to be effective but has drawbacks of inducing hypoglycaemia and hypokalaemia.[14] However, the limited research on localised insulin treatment has shown to overcome this and is a promising future therapeutic for the treatment of wounds.[15]

The aim of this systematic review and meta-analysis was to explore the efficacy and safety of localised insulin application on acute and chronic wounds, and its ability to promote wound healing. Although some studies have previously addressed this, including a review by Sridharan and Sivaramakrishnan,[15] these usually included diabetic patients. This study specifically focused on non-diabetic adults, since there is a lack of evidence exploring this population despite prevalence of different kinds of wounds in this group.

2 | MATERIALS AND METHODS

2.1 | Literature search

The search strategies recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines[16] were used for this review. Studies were searched systematically from inception to 10 July 2022 by two reviewers (Zunira Areeba Bhuiyan and Zubair Ahmed) using the Embase, Ovid MEDLINE and PubMed electronic databases. A filter was applied to the PubMed database to narrow search results to include only clinical trials and randomised controlled trials (RCTs). Key terms used for this search included: 'insulin', 'adult', 'human' AND 'wound healing OR ulcer healing' where Boolean operators were utilised (full search strategy available in Supporting Information Table S1). Referencing lists of reviewed articles were further screened for additional relevant studies.

2.2 | Eligibility criteria

The inclusion and exclusion criteria for this review is provided in Supporting Information Table S2. No limitations were set with regard to publication date, where all studies published before the date of search (10 July 2022) were eligible for inclusion. However, non-English studies were excluded. Studies were limited to RCTs only, as these are classed as the highest level of primary research in the hierarchy of evidence.[17] Any conflicts on eligibility criteria were resolved through discussion with the senior author (Zubair Ahmed).

2.3 | Data extraction

Studies retrieved by the literature search were initially screened using their title and abstract by two independent reviewers (Zunira Areeba Bhuiyan and Oluwasemilore Adebayo). Subsequently, relevant studies underwent full-text analysis to determine eligibility, where any disagreements were resolved through discussion with the senior author (Zubair Ahmed). Data extraction was then performed, where the following information were extracted onto a spreadsheet: study characteristics (first author, year, title, country of study, inclusion and exclusion criteria, intervention and control protocols, outcome measures and results), population characteristics (sample size, age, gender, wound location [upper or lower limb]) and outcome measures. Where information was unavailable, the tables were left blank as no assumptions were made. The primary outcome extracted for this review was the rate of healing of the wound (mm^2/day). Secondary outcomes extracted include healing time (days), reduction in wound area (cm^2), safety evaluation and adverse effects of insulin and quality of life (QoL). Trials with multiple treatment arms were included and analysed in our systematic review. However, data from the best treatment effects in these trials were used to compare studies for the meta-analysis.

2.4 | Risk of bias

The risk of bias of the included studies were assessed using the Revised Cochrane Risk-of-Bias Tool for Randomised Trials (RoB 2)[18] by two independent reviewers (Zunira Areeba Bhuiyan and Oluwasemilore Adebayo). Five bias domains were considered including: bias arising from the randomisation process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement

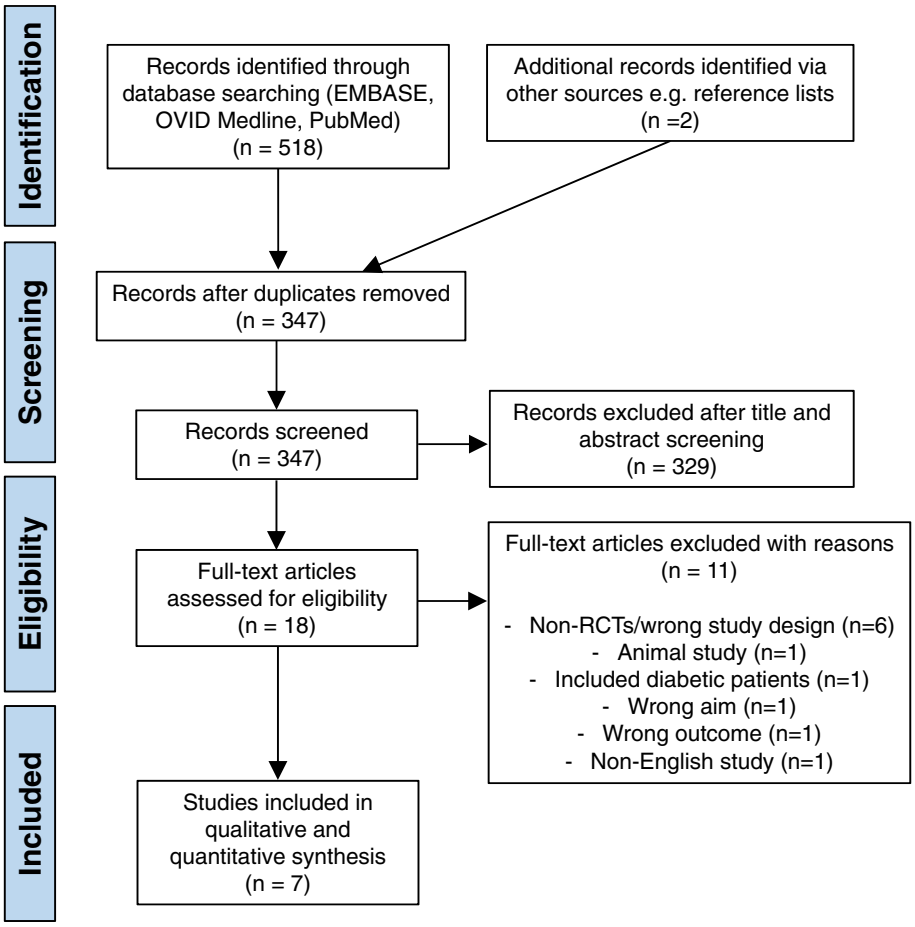

FIGURE 1 PRISMA study selection flow chart.

of the outcome and bias in selection of the reported results. Risk-of-bias judgements were scored as either 'low', 'some concerns' or 'high' for each domain. The overall risk of bias was also determined where individual scores of the domains were considered. Any disagreements were resolved through discussion with the senior author (Zubair Ahmed).

2.5 | Statistical analysis

Assessment of heterogeneity was performed by examining the differences across studies for methodological heterogeneity. We used Review Manager (RevMan 5.3, Cochrane Informatics & Technology, London, UK) to determine the Q and I^2 statistics (in percentages) to establish variation between the studies attributed to heterogeneity. A meta-analysis was conducted in RevMan 5.3 (Cochrane Informatics & Technology) using the dichotomous data function, employing a random effects model.

3 | RESULTS

3.1 | Study selection

The literature search initially yielded 518 studies with an additional two studies identified through other sources (i.e., reference lists). Overall, 347 studies were extracted for screening after removal of duplicate studies. Then, 329 records were excluded following the initial title and abstract screening having applied the proposed inclusion and exclusion criteria. A further 11 studies were excluded after a full-text analysis, with reasons provided on the PRISMA flow chart in Figure 1. Subsequently, a total of seven studies were included in the qualitative and quantitative synthesis of data in this review.

A RCT by Martínez-Jiménez et al.,[19] which explored the use of insulin on wound healing in non-diabetic patients, was excluded from being selected in this systematic review. This is because the control and insulin intervention were applied in different areas of the same wound of individual patients. Therefore, the effects of the control and insulin intervention may be compromised, and interpretation of the data may be biased.

3.2 | Study characteristics

The seven RCTs included in this review were published between August 2009 and November 2021.[20–26] These were undertaken in either of the following countries: China, Egypt, India or Iran. Four of the seven studies had a two-armed RCT design, where the therapeutic group explored insulin as a treatment for wound healing and the control group had a standard saline placebo.[20–23] Two of the seven

TABLE 1 Overview of study characteristics.

Study and year	Country of study	Inclusion criteria	Exclusion criteria	Intervention	Control group	Outcome measures and results
Sun et al., 2021	China	Acute wounds (burns or crush wounds) Age ≤ 75 years old	Diabetes mellitus; pregnancy; patients with blood systemic illnesses; patients with blood systemic diseases (immunosuppressive treatment was administered to the patients) Wounds that are complicated (e.g., bleeding or infection) Medications that may impact the study's outcome Cardiovascular illnesses; peripheral artery disease; renal and hepatic failure	For each 10 cm^2 of wound, 10 units of insulin injected in solution with 1 mL 0.9% saline	Normal saline	Rate of wound healing (mm^2/day): $p = 0.008$. Healing time (days): $p = 0.39$. Granulation tissue coverage rate (%): $p < 0.05$. Thickness of granulation tissue (mm): $p < 0.05$. Safety evaluation: no adverse events were recorded.
Singh and Pawar, 2020	India	Patients who had completed a multidrug treatment for leprosy, bacterial index (BI) ≤ 1, no lepra reactions, ankle-brachial pressure index ≥ 0.8, and wounds less than 10 cm^2 with a negative surface swab	Patients older than 70 years; smokers; and those with hypertension, osteomyelitis, immunodeficiency, vascular insufficiency, uncontrolled diabetes, and/or any signs or symptoms of secondary bacterial ulcer infection Patients who are pregnant	10 units (0.1 mL) of topical insulin in 1 mL of normal saline twice daily over treated areas.	Normal saline	Rate of healing (cm^2/week): $p < 0.0001$. Healing time (days): $p = 0.02$. Wound area reduction: $p < 0.0001$. Physician Global Assessment of efficacy (PGA) score: decreased in both groups, statistically significant decrease in insulin group. Dermatology Life Quality Index (DLQI) score: statistically significant improvement in both groups, more in insulin group.
Stephen et al., 2016	India	Patients admitted to an acute care facility who had a Grade 2 or Grade 3 pressure ulcer	Patients with immunodeficiency, diabetes mellitus, pregnancy, osteomyelitis, and peripheral vascular illness	Insulin dressing—twice a day for 7 days, 1 U/cm^2 wound area	Normal saline dressing	Reduction in wound area: $p < 0.01$ in insulin group, $p = 0.566$ in control group. Pressure Ulcer Scale for Healing (PUSH) score: $p < 0.01$ in insulin group, $p = 0.475$ in control group.
Rezvani et al., 2009	Iran	Patients with acute (crush wounds, burns) and chronic wounds (pressure ulcers) of the upper and lower extremities	Patients with uncontrolled wound bleeding, severe infection (as determined by the presence of visible pus, wound exudate, redness, or warmth of the wound border; no wounds had visible pus or exudate), immunodeficiency, age > 75 years, or chronic medical conditions (such as diabetes mellitus)	10 units (0.1 mL) of insulin crystal in solution with 1 cc saline 0.9% for each 10 cm^2 of wound, twice daily topical spray	Saline spray	Wound healing rate (mm^2/day): $p = 0.029$. Healing time (days): $p = 0.928$. Safety evaluation: no adverse events or reactions.

TABLE 1 (Continued)

Study and year	Country of study	Inclusion criteria	Exclusion criteria	Intervention	Control group	Outcome measures and results
Wang et al., 2016	China	Patients aged ≥18 years but <75 years Patients were conscious and were treated for the first time Informed consent was obtained	Patients with serious infection, shock, unmanageable intense pain, inhalation pulmonary injury, coagulation disorders, severe organ dysfunctions such as heart, liver, and kidney Patients with skin transplantation failure, and serious diseases Pregnant patients and patients with an estimated lifespan of <12 months	(i) Large-dose insulin group: 1.0 μ long-term suspended zinc insulin locally injected (ii) Low-dose insulin group: 0.1 μ long-term suspended zinc insulin locally injected	Normal saline injection	Wound surface area (%): low-dose group significantly decreased than other groups, $p < 0.05$. Healing time (days): $p = 0.025$. Wound infection cases (%): $p < 0.001$.
Attia et al., 2014	Egypt	Patients with acute wounds (burns or crush wounds) or chronic wounds (pressure ulcer)	Patients whose age > 75 years, smokers, patients with immunosuppression, cardiovascular diseases, diabetes mellitus, any chronic debilitating disease, low serum zinc level, complicated wounds (e.g., bleeding or infection), history of abnormal scar formation and/or previous or current medications likely to affect the outcome of the study	(i) 10 units (0.1 mL) regular insulin in solution with 1 cc saline 0.9% for each 10 cm^2 of wound (ii) Sterile aqueous zinc chloride solution	Sterile 0.9% saline (0.9% NaCl), twice daily	Healing rate (mm^2/day): $p < 0.001$ in insulin group, significantly higher in acute wounds ($p < 0.001$), in those ≤40 years ($p = 0.004$), and in upper body wounds ($p = 0.015$). Quality of life: improved most in insulin group, $p = 0.015$.
Zeng et al., 2016	China	Patients who underwent free skin flap transplantation for simple deep burns	Presence of co-existing diseases including acute lung injury, severe infections, malnutrition, diabetes, scarring tissues, other severe underlying diseases and failed free flap transplantation	(i) Low-dose insulin group: 0.5 units regular insulin injections (ii) Medium-dose insulin group: 1.0 units regular insulin injections (iii) High-dose insulin group: 2.0 units regular insulin injections	(i) Saline control: saline injections. (ii) Blank control: no local subcutaneous drug injections.	Wound healing rate (%): $p < 0.001$. Healing time (days): $p < 0.001$. Histological observation: low-dose insulin group had a reduced number of inflammatory cells, increased fibroblast count, increased capillary components, and partial regenerated epithelial tissue.

studies had a three-armed RCT design.[24,25] In the study by Wang et al.,[24] patients were divided into the following three groups: (i) low-dose insulin, (ii) high-dose insulin and (iii) saline control. The groups in the study by Attia et al.[25] included: (i) regular insulin, (ii) aqueous zinc chloride solution, and (iii) saline control. The RCT by Zeng et al.[26] on the other hand had five-arms in its design, which consisted of: (i) low-dose insulin, (ii) medium-dose insulin, (iii) high-dose insulin, (iv) saline control group and (v) blank control group (no local subcutaneous drug injections). A detailed description of the study characteristics is provided in Table 1, with information regarding the inclusion and exclusion criteria, intervention and control groups, and outcome measures and results.

3.3 | Patient characteristics

Various information on patient demographics were provided in all of the included studies, which are detailed in Table 2. This includes age, gender (male) and location of the wound (either upper limb or lower limb), as well as the number of participants involved. The table is left blank where no information was provided in the studies.

Five studies assessed the primary outcome of exploring the rate of healing in wounds due to insulin intervention.[20,21,23,25,26] However, only four were included in the quantitative analysis as shown in Table 3.[20,21,23,25] This was due to the fifth study using a different parameter to explore the rate of healing-percentage at Days 7 and 14 instead of mm^2/day.[26] Despite omission, the study showed a statistically significant difference favouring the insulin groups, where $p < 0.001$ at both Days 7 and 14. Table 3 displays the mean rate of healing for each study with the standard deviation and *p*-value.

3.4 | Results of individual studies—Primary outcome

Three studies reported wound healing rate in mm^2/day,[20,23,25] whilst one study reported the rate in cm^2/week.[21] The mean rate of wound healing ranged from 46.1–53.5 mm^2/day, with one study reporting healing rate of <1 mm^2/day.[21] To assess the strength of evidence present, a meta-analysis with these four studies (Figure 2) showed an overall significant weighted mean improvement of rate of healing by 11.84 mm^2/day (95% CI: 0.64–23.04; $p = 0.04$; $I^2 = 97\%$) favouring the insulin treatment group.

3.5 | Results of individual studies—Secondary outcomes

Five of the included studies explored the time taken for wounds to heal, measured in days. These data have been collated in Table 4, where three studies concluded a significant difference found in the insulin group compared to the control group.[21,24,26] However, the studies by Sun et al.[20] and Rezvani et al.[23] established there are no discernible disparity between the groups, where the *p*-values are 0.39

TABLE 2 Overview of patient demographics in included studies.

					Location of wound (*n*, %)	
Study	**Insulin/control groups**	**Number of participants**	**Age (years)**	**Gender, male (*n*, %)**	**Upper limb**	**Lower limb**
Sun et al., 2021	Insulin group	146	47.4 ± 5.27	83 (56.8%)	48 (32.9%)	57 (39.0%)
	Control group	150	45.4 ± 5.46	62 (41.3%)	53 (35.3%)	45 (30.0%)
Singh and Pawar, 2020	Insulin group	12	41.3 ± 2.11	M:F ratio—1.88		
	Control group	19	40.9 ± 3.02	M:F ratio- 1.71		
Stephen et al., 2016	Insulin group	25	43.4 ± 14.41	21 (84.0%)		
	Control group	25	41.6 ± 16.53	19 (76.0%)		
Rezvani et al., 2009	Insulin group	23	42.0 ± 17.37	15 (65.2%)	4 (17.4%)	19 (82.6%)
	Control group	22	39.2 ± 16.76	14 (63.6%)	4 (18.2%)	18 (81.8%)
Wang et al., 2016	Insulin group—low dose	23	50.3 ± 14.60	17 (73.9%)		
	Insulin group—high dose	19	48.7 ± 13.60	13 (68.4%)		
	Control group	16	49.8 ± 12.50	11 (68.8%)		
Attia et al., 2014	Insulin group	30			13 (43.3%)	17 (56.7%)
	Zinc chloride group	30			13 (43.3%)	17 (56.7%)
	Control group	30			15 (50.0%)	15 (50.0%)
Zeng et al., 2016	Insulin group—low dose	33	53.2 ± 12.20	21 (63.6%)		
	Insulin group—medium dose	33	54.5 ± 13.50	20 (60.6%)		
	Insulin group—high dose	33	52.7 ± 12.60	21 (63.6%)		
	Control group (saline)	33	51.3 ± 11.40	20 (60.6%)		
	Blank control group (no drug)	33	52.4 ± 10.30	19 (57.6%)		

TABLE 3 Table of primary outcome (rate of healing in insulin and control treated groups).

	Insulin Group			Control Group			
Study	Number of pts	Mean	Standard deviation	Number of pts	Mean	Standard deviation	*p*-value
Sun et al., 2021 (mm^2/day)	146	46.7	±19.5	150	30.5	±14.1	0.008
Rezvani et al., 2009 (mm^2/day)	23	46.1	±21.6	22	32.2	±14.6	0.029
Attia et al., 2014 (mm^2/day)	30	53.5	±12.1	30	36.1	±5.70	<0.001
Singh and Pawar, 2020 (cm^2/week)	12	0.61	±0.31	19	0.14	±0.42	<0.0001

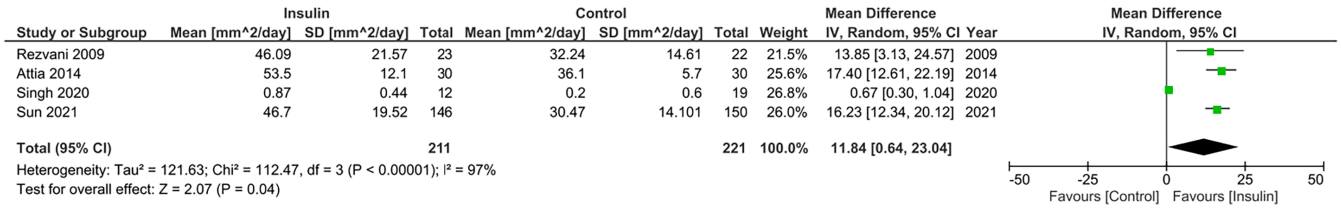

FIGURE 2 Meta-analysis for the rate of wound healing in insulin- and control-treated groups.

TABLE 4 Table showing healing time in insulin and control treated groups.

	Insulin Group			Control Group			
Study	Number of pts	Mean	Standard deviation	Number of pts	Mean	Standard deviation	*p*-value
Sun et al., 2021	146	34.4	±14.2	150	32.7	±9.4	0.39
Singh and Pawar, 2020	12	31.5	±17.6	19	44.3	±16.2	0.02
Rezvani et al., 2009	23	41.7	±20.6	22	43.5	±22.9	0.928
Wang et al., 2016	23	16.4	±6.3	16	24.1	±7.5	0.025
Zeng et al., 2016	33	18.2	±3.3	33	26.6	±4.5	<0.001

and 0.93, respectively. It should be noted that the values in Wang et al.[24] and Zeng et al.[26] on the table are both using data from the low-dose insulin groups (both studies had multiple intervention arms) as these expressed the biggest difference in results.

A meta-analysis exploring healing time was carried out using these five studies, as expressed in the forest plot in Figure 3. The test for overall effect shows that the *p*-value is 0.07 (IV: −5.40; 95% CI: −11.28 to 0.48; $I^2 = 89\%$); hence, it could be concluded that this outcome measure does not exhibit a major clinical difference between the groups.

Another outcome explored is the reduction in wound area (cm^2) due to insulin. The study by Stephen et al. showed that the overall mean wound area decreased from 11.79 ± 8.97 cm^2 (Day 1) to 11.43 ± 9.06 cm^2 (Day 7) in the control group ($p = 0.566$) and from 9.61 ± 6.39 cm^2 (Day 1) to 6.24 ± 4.33 cm^2 (Day 7) in the intervention group ($p < 0.01$).[23] This shows a significant difference favouring the insulin group to effectively reduce wound area. Similar results were expressed in Singh and Pawar,[21] where the *t*-test value for reduction in mean wound area was 4.96 ($p < 0.0001$), indicating a highly significant reduction.

Safety evaluation and adverse effects of insulin on wounds are also explored in some studies. Rezvani et al. concluded that no significant adverse drug events or reactions were observed; this included hypoglycaemia, hypokalaemia, hypoaminoacidemia, vertigo and headache.[23] Similar findings were established in Sun et al. where rates of wound infection, malnutrition, osteomyelitis and septicaemia were not statistically different between groups ($p > 0.05$).[20] However, in the study by Wang et al., wound infection cases were noted to be significantly lower in the insulin groups where $p < 0.001$.[24] This study also showed that infection rates were further reduced in the low-dose insulin group (4.3%) compared to the high-dose group (15.8%).[24] Sun et al. observed a significant difference in the occurrence of bleeding of wound (6.8% vs. 10.7%) and suppurative wounds (1.4% vs. 5.3%) between the two groups ($p < 0.05$).[20]

Attia et al. assessed the QoL using the 'Effect of Pain from the Wound on QoL' questionnaire, which included the following parameters: physical non-functioning, role limitation due to physical health, role limitation due to emotional problems, wound status improvement, social non-functioning, pain, and bad general health.[25] This showed a significant difference between the before and after for all questions in the three groups ($p < 0.001$ for all), hence no notable improvement due to insulin. The correlation between the improvement of all seven parameters and the rate of wound healing in the

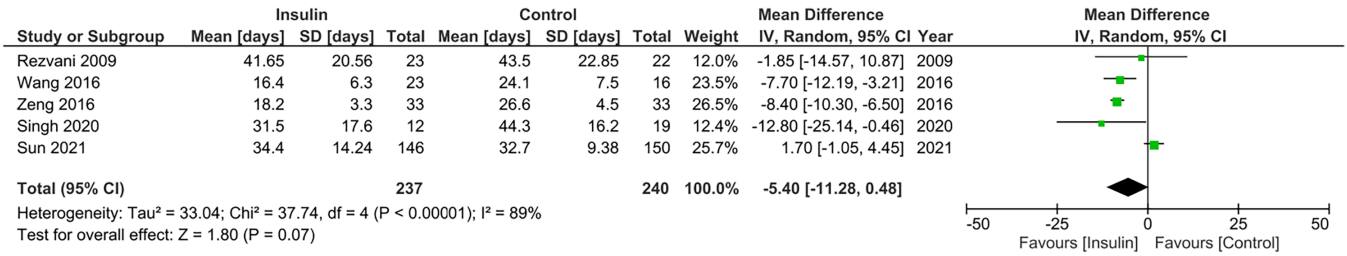

FIGURE 3 Meta-analysis for healing time in insulin- and control-treated groups.

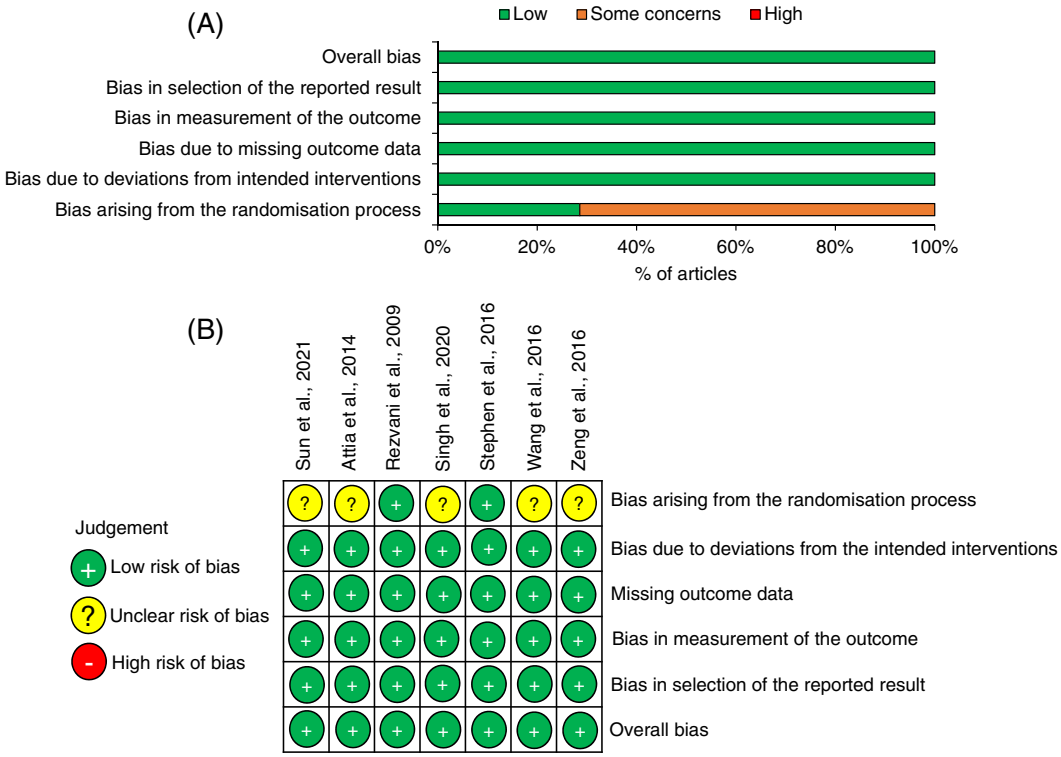

FIGURE 4 Risk of bias analysis. (A) Summary graph and (B) bias in individual studies.

study groups were also explored, where pain and bad general health had markedly made the most significant difference ($p < 0.001$).[25] The impact of chronic leg ulcers on QoL was explored in Singh and Pawar, where the Dermatology Life Quality Index (DLQI) scoring system was used.[21] In the insulin group, DLQI scores decreased notably from 14.72 ± 6.29 at baseline to 5.37 ± 4.49 at 12 weeks post-intervention ($p < 0.0001$; 95% CI: −12.60 to −6.10), whereas in the placebo group, scores decreased from 14.49 ± 5.98 to 10.09 ± 6.53 ($p = 0.037$; 95% CI: −85,198 to −0.2802).[21] Although the difference in QoL is more distinctive in the insulin group, both groups display a statistical difference in the before and after.

3.6 | Risk of bias in studies

The risk of bias of the seven included RCTs are summarised in Figure 4A, with bias in individual studies depicted in Figure 4B. As shown, 70% of studies had an unclear risk of bias arising from the randomisation process.[20,21,24–26] This was primarily due to no information given in the studies regarding the randomisation of allocation sequence and/or the concealment of allocation sequence until participants were enrolled and assigned to interventions. For the other domains in the RoB 2 tool, the studies were judged as having a low risk of bias. Consequently, the overall risk of bias is low for all of the included studies.

4 | DISCUSSION

4.1 | Review findings

We aimed to conduct a systematic review of the current literature regarding local insulin administration for wound healing and its efficacy and safety in non-diabetic adults. The result of the primary outcome suggests that insulin administration is associated with a faster rate of healing compared to patients who did not receive

insulin. This can be explained by the findings from Liu et al. that observed that, in wounds, insulin facilitates migration of keratinocytes via its receptors, which promotes re-epithelialisation, hence speeding up the process of healing.[27] Multiple animal studies have investigated this theory, including Apikoglu-Rabus et al., where topical insulin was found to enhance wound healing by shortening the duration of epithelialisation in non-diabetic rats.[28] Furthermore, studies show that local insulin stimulates the formation of new micro-vessels, where the increased blood flow can encourage primary healing.[19]

However, this review has also established that the average wound healing time was not significantly different in the insulin group compared to the controls. This somewhat contradicts the primary outcome finding; if the healing rate was faster in the insulin group, it should take fewer days for the wound to heal. This may be due to a possibility of the results being underpowered and a larger sample size would be required in future studies to fully explore this. Another potential explanation for this discrepancy may be that the initial wound area in participants from the insulin group was bigger compared to the controls, thus taking longer to heal. However, due to the randomised nature of the RCT study design, this selection bias is theoretically less likely to have occurred in the included studies.[29] Having said that, four of the five studies that explored the healing time outcome were in fact judged as having an unclear risk of bias in the randomisation process according to the RoB 2 tool.[20,21,24,26] Hence, this premise cannot be disregarded for potentially introducing selection bias, which may help to explain the non-significant wound healing time. Moreover, Rezvani et al. reported that the initial wound area correlates with wound healing rate, where larger wounds heal at a faster pace.[23] This observation further supports this theory, but additional research is warranted to further explore this concept and correlation.

Other secondary findings of this review concluded that insulin administration is a potentially safe procedure with no adverse events or side-effects reported. However, the absence of reported risks does not necessarily imply that insulin administration is safe and further high-quality studies are required to reach definitive conclusions. Nonetheless, low levels of insulin administration does minimise the risk of wound infections and improves healing.[24] This finding is also reported in another study where, compared to normal saline, topical insulin increased bacterial clearance rate in wounds, thereby reducing the chance of wound infection.[30] Studies show that sustained skin wounds are likely to become contaminated with bacteria that can encourage unfavourable complications; thus, it is important to prioritise methods to reduce this.[7,13] Insulin is therefore a potential intervention to explore, as it can directly combat infections and reduce complication rates.

Another outcome studied in this systematic review is QoL, which drastically improved with wound healing irrespective of intervention (insulin or control).[21,25] It can be argued that as the rate of healing in the insulin groups are relatively faster, it may have an accelerated impact on QoL and hence can be deemed as the more favourable treatment.

4.2 | Role of insulin

Insulin is a peptide hormone that has the ability to alter inflammation by promoting anti-inflammatory cytokines (such as IL-10, IL-4 and VEGF) as well as decreasing expression of pro-inflammatory cytokines (such as IL-6, IL-1β, IL-12 and TNF-α) via suppression of NFkβ P50/P65.[11] Research shows that NFkβ P50/P65 inactivation by insulin can also induce glucose uptake by cells and store it as glycogen.[11] This reduces the risk of a hyperglycaemic environment, which is pro-inflammatory and correlates to vascular damage and reduced tissue oxygenation.[11,31] This in turn reduces inflammation and promotes wound healing.

Insulin is also shown to stimulate lipogenesis (fatty acid synthesis), which can further supress TNF-α-mediated inflammation.[11,30] Additionally, protein synthesis is stimulated by insulin that promotes cell growth and differentiation, whilst proteolysis is inhibited via inactivation of the Fork head box protein O1 (FOXO) pathway.[11] This contributes to the acceleration of wound healing.

4.3 | Previous studies

It is well established that insulin has beneficial effects on wound healing, as well as its role in regulating glucose, lipids and proteins.[32] Most of these previous studies, however, have focussed on wounds in experimental animal studies and, more recently, diabetic patients. A limited number of primary research has been undertaken targeting the non-diabetic population, where this systematic review is the first compilation of RCTs exploring this group.

In 2017, a similar systematic review and meta-analysis was undertaken by Sridharan and Sivaramakrishnan, where the efficacy of topical insulin in wound healing was explored (inclusive of diabetic and non-diabetic patients).[15] Overall, findings concluded that no significant differences were observed in the healing rate between the study groups ($p = 0.96$). A sub-group analysis for the non-diabetic population was also undertaken (three studies were included),[23,25,33] which similarly concluded no significant difference (IV = 0.2; 95% CI: −1.46 to 1.87; $p = 0.81$). This is contradictory to the results obtained in our systematic review, where a significant difference was established regarding healing rate. This discrepancy in findings could be subjected to multiple factors, including the limited number of studies in the prior review as well as the exclusion of new emerging RCTs post-2017 when the study was completed. Thus, it could be assumed that the conclusion of our review is scientifically more representative and accurate.

4.4 | Clinical implications

Our systematic review explored the potency of insulin administered locally—this could be by means of topical application (via ointment or insulin-soaked dressing), irrigation (washing wound with an insulin solution) or injection at site. Local administration has multiple benefits

of directly targeting the cells in the wound to enhance healing; it generally lowers the dosage of insulin needed compared to systemic administration too, ultimately reducing the cost.[15] Furthermore, systemic insulin has downsides of inducing hypoglycaemia and hypokalaemia,[14] which is not seen in local administration according to this review. A thorough investigation of the different local administration techniques is warranted to explore the most beneficial and effective method of providing insulin in the clinical setting, as this is not reviewed in our study. Factors such as efficacy, cost, ease of use, adverse events and patient preference should also be considered here.

It is important to consider the optimum dosage of insulin required for wound healing in practice. The RCTs by Wang et al. and Zeng et al. in this review explored this, where there were at least two different dosages of insulin injections investigated (0.0001 mL vs. 0.001 mL and 0.005 mL vs. 0.01 mL vs. 0.02 mL, respectively).[24,26] These studies conclude that the low-dose insulin groups in effect had better outcomes where there was a significant improvement in healing rate, healing time, re-epithelialisation and reduced rates of infection compared to the higher doses.[24,26] The median dose in Zeng et al. was deemed more effective than the higher dose, further supporting this finding.[26] Additionally, low-dose insulin is believed to improve insulin resistance, promote insulin secretion and increase insulin sensitivity.[24] In practice, this is useful where a low dose can further reduce costs and the likelihood of unwanted side-effects, whilst still being effective.

With this in mind, having assessed the results from the meta-analysis, the studies by Sun et al. and Attia et al. showed more of a significant difference in the rate of healing than the other studies.[20,25] However, both used 10 units (0.1 mL) of insulin as their intervention, which is considerably higher than the noted optimum doses mentioned. This is due to the differences in insulin administration in these studies (insulin spray and insulin-soaked sterile gauze, respectively, vs. insulin injection in the prior studies). Hence, as previously stated, it is a priority to explore the different routes of administration, so optimum doses and their efficacy can be assessed with each procedure. It may also be worthwhile to investigate the use of different agents alongside insulin, such as zinc as reviewed in the study by Attia et al.,[25] where it may be regarded as more effective than insulin therapy alone.

IGF-1 is a hormone that has a similar molecular structure to insulin where it can bind to the insulin receptor.[23,34] This potentially allows it to be an agent of interest when exploring wound healing therapeutics. However, studies show that IGF-1 activates the insulin receptor at approximately 0.1 times the potency of insulin, hence is not as effective.[35,36] Nevertheless, insulin has its own benefits, where it is deemed as a cost-effective drug, which is globally available in large-scale production.[13] Cost of other growth factors currently used in wound healing (e.g., epidermal growth factor, transforming growth factor beta and platelet derived growth factor, etc.) can range from 1500 to 10,000 USD per mg, while insulin is a much cheaper alternative.[13] Furthermore, research on insulin has been undertaken for decades, where its mechanism, safety and long-term consequences are relatively well understood.

It is important to consider the characteristics of different types and severity of wounds and appreciate that the differences in their pathophysiology and anatomy may affect wound healing time and rate. For example, superficial burns are predicted to heal faster than deep burns. This review, however, did not acknowledge these differences when analysing the data for the meta-analysis. This was primarily due to the limited number of studies available; further research is warranted to explore insulin therapy for specific wound types, so an insulin regime can be aptly tailored clinically for each wound.

Overall, the clinical usage of insulin for wound healing appears to be a promising concept. It has the potential to be an appropriate alternative or addition to many of the therapies available in practice today. However, despite supporting literature, it has been challenging to standardise insulin therapy for practical application.[19] A better understanding of its mechanism of action in the context of wound healing, appropriate dosage and optimal route of delivery is required. Large-scale, prospective RCTs inclusive of different types of wounds are warranted to explore this, where future policies can be made to allow insulin therapy to be clinically available for wound healing.

4.5 | Risk of bias

The leading bias observed in the RCTs using the RoB 2 tool were the bias arising from the randomisation process in five of the included studies. This was primarily due to a lack of: (i) randomisation in the allocation sequence, and/or (ii) concealment of allocation sequence until participants were enrolled and assigned to interventions. To overcome the former, details of the randomisation process are warranted, where allocation methods such as repeated coin-tossing, throwing dice, shuffling cards or using computer-generated random numbers would suffice as a successful randomisation. For the latter, a form of remote or centrally administered method to allocate interventions to participants is needed, where the process of allocation is controlled by an external unit or organisation that is independent of the enrolment personnel. This could be done by sequentially numbered drug containers (with insulin) prepared by an independent pharmacy, or using sequentially numbered, opaquely sealed envelopes to conceal allocation.

4.6 | Limitations

Limitations of this review are mostly due to the restricted number of RCTs available in the topic area, especially those with a focus on the non-diabetic population. Nevertheless, all of the studies involved were completed to a very high standard. Despite some studies having an unclear risk of bias in the randomisation process, the risk of bias overall for all RCTs were deemed low. However, the studies themself had some limitations. This included the small sample size where it makes it difficult to determine if an outcome is a genuine finding or due to chance—a small population increases the likelihood of false-positive results. The study duration was also relatively short for some

RCTs, where the true effect of insulin in the long-term were not explored. Confounding variables were also not considered for potentially influencing outcomes. The single setting of the studies also limits the variety of wounds investigated, which may reduce the generalisability of the results overall. Additionally, there is a possibility that the findings may not be representative, as all of the studies took place in low- and middle-income countries. However, it can be argued that it should not be challenging to translate these findings to the non-diabetic population in higher-income countries, as the mechanism of wound healing and insulin therapy should be the same for everyone regardless of location. Furthermore, high-quality RCTs with a larger and longer design protocol are warranted to fully establish the efficacy of topical insulin for wound healing.

Our review may include publication bias due to the exclusion of non-English language articles in the search strategy. This could result in valuable findings being dismissed, especially since the pool of data available on the topic is already limited. The further use of filters to limit search results in the PubMed database could potentially cause this too.

Despite extracting data on patient characteristics from the RCTs, sub-group analyses were not undertaken in our review to explore association of these factors and the outcomes. This was primarily due to the limited number of studies providing this information in full. However, for future studies, it would be interesting to see if these characteristics have any correlation with wound healing, and how. Other characteristics that could also be potentially explored are the cause of wound (crush injury, penetrating wounds, burns, metabolic wound, etc.), location of wound (upper/lower limb, torso, etc.) and classification of wound (clean, contaminated, dirty, etc.). This would better our understanding of factors affecting wound healing, and whether insulin is beneficial for certain types of wounds or not. This would further help guide policies into whether insulin should be a standardised treatment administered for a specific type of wound in clinical practice. Overall, the strengths of this study include its comprehensiveness in methodology and data search, reproducibility of results and generalisability of findings in the clinical setting.

5 | CONCLUSION

In conclusion, our systematic review and meta-analysis shows that localised insulin administration in non-diabetic adults was beneficial for wound healing, with an improvement in the rate of healing, reduction in wound area and QoL. The use of insulin was also observed to have no adverse events or side-effects. Furthermore, high-quality RCTs are warranted to fully explore the effects of localised insulin on different types and severity of wounds, also considering factors such as age, gender, cause, location and classification of wounds. This would further support the development of an appropriate insulin regime, considering optimum dosage and appropriate route of administration, in the clinical setting.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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