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USE OF HUMAN INSULIN IN WOUND HEALING IN PATIENTS WITH DIABETES: MECHANISMS AND FUTURE PERSPECTIVES. *REVIEW*

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Abstract: This review explores how insulin, in addition to participating in glycemic control, acts directly on wound healing, especially in diabetic patients. Chronic hyperglycemia damages skin proteins (such as collagen and elastin) through the formation of advanced glycation end products (AGEs), impairing tissue recovery. This damage aggravates chronic inflammation, reduces angiogenesis, and compromises cellular repair function. Studies show that locally administered insulin via drip can reverse some of these effects, accelerating healing without significant risks of systemic hypoglycemia. The research highlights the molecular mechanisms that explain the difficulty of healing in diabetics and points to a reassessment of the therapeutic potential of topical insulin. The focus is on understanding and exploring how insulin acts in the healing of chronic wounds in diabetic patients. Methods: This review is based on a critical survey of the literature indexed in PubMed and MEDLINE (2000–2025), with a search guided by descriptors such as "topical insulin," "diabetic wound healing," and "molecular mechanisms of insulin in tissue repair." Clinical trials, preclinical studies (in vitro and in animal models), systematic reviews, and conceptual articles addressing the local effects of human insulin on skin repair were included. Studies restricted to systemic insulin therapy were excluded. The selection prioritized biological relevance, methodological soundness, and contribution to the understanding of cellular mechanisms and clinical implications. The findings were organized into four axes: pathophysiology of diabetic healing, molecular signaling of insulin, clinical evidence, and translational perspectives. Results: Clinical trials have explored interventions for hard-to-heal wounds, targeting molecular, cellular, and tissue processes such as inflammation, angiogenesis, and oxidative stress. Despite progress, there is no single effective solution for all cases. Personalized strategies, combining therapies according to the cause of the wound, show promise for improving outcomes. Continued research and standardized protocols are essential to advance the treatment of these complex injuries. Discussion: The ability of local insulin to simultaneously modulate oxidative stress, chronic inflammation, and angiogenesis positions it as a unique intervention in the scenario of therapies for diabetic ulcers, where isolated approaches often fail. Unlike recombinant growth factors, whose high cost limits access in public systems, regular human insulin is generic, registered in the SUS (Brazilian Unified Health System), and can be incorporated into low-cost formulations, such as hydrogels. However, the lack of consensus on optimal concentration, frequency of application, and outcome criteria makes it difficult to compare studies and implement standardized clinical protocols. Conclusion: Human insulin shows potential to accelerate healing in diabetics, with robust evidence of cellular mechanisms and reduced complications. Advances in pharmaceutical formulations and combination therapies may overcome challenges such as standardization and safety, integrating it into chronic wound management.

Keywords: Diabetes mellitus; Insulin; Tissue repair; Topical treatment; Cellular mechanisms.

INTRODUCTION

Current figures reveal a worrying increase: more than 10% of adults live with diabetes. In several countries, this rate is

even higher, reaching 20% or more of the adult population. Since the first edition of the report in 2000, the number of adults with diabetes (between 20 and 79 years old) has more than tripled: from 151 million (4.6% of the world population) to 537 million (10.5%) today. If effective measures are not taken, it is estimated that 643 million people (11.3% of the population) will have diabetes by 2030. If the current trend continues, this number could reach 783 million (12.2%) by 2045 (INTERNATIONAL DIABETES FEDERATION, 2021).

Diabetic foot ulcers represent one of the most microvascular complications

devastating consequences of diabetes mellitus, often progressing to deep infections that require prolonged hospitalization and, in uncontrolled cases, invasive procedures such as surgical debridement or even lower limb amputation—accounting for up to 85% of non-traumatic amputations in adults (BOULTON et al., 2005; EDMONDS et al., 2021). This cascade of adverse events imposes high morbidity and mortality and significant healthcare costs on health systems (WANG et al., 2022).

The difficulty in healing observed in diabetic individuals is related to a series of interconnected pathophysiological factors, such as high blood glucose levels, compromised immune system, damage to small blood vessels, and oxidative stress (BURGESS et al., 2021; PASUPULETI et al., 2020).

The molecular understanding of these effects has been consolidated by studies that have demonstrated activation by the PI3K/Akt and MAPK/ERK signaling pathways in keratinocytes, fibroblasts, and endothelial cells, promoting cell migration, angiogenesis, and extracellular matrix synthesis (BREM; TOMIC-CANIC, 2007). Although recent systematic reviews corroborate the experimental benefits of local insulin—such as modulation of inflammation and promotion of angiogenesis (WANG et al., 2020)—the guidelines of the International Working Group on the

Diabetic Foot (IWGDF, 2019) do not include it as a formal recommendation due to the heterogeneity of protocols and the absence of robust multicenter trials, maintaining its use as an off-label option in specific contexts.

This review focuses exclusively on the role of topical human insulin in diabetic wound healing, without addressing combination therapies or other physical interventions. Combined approaches, including biophysical therapies such as microcurrents, are the subject of parallel research and will be presented in future studies. This manuscript is limited to reviewing the isolated use of topical insulin.

EPIDEMIOLOGY AND IMPACT OF DIABETIC LESIONS

Diabetic foot ulcers are a serious public health problem, with an estimated lifetime risk of between 15% and 25% among individuals with diabetes (BOULTON et al., 2008). In Brazil, they are the leading cause of hospital admissions among the diabetic population and account for approximately 70% of non-traumatic lower limb amputations (BRASIL, 2019). After initial healing, recurrence is high—about 40% in one year and up to 65% in five years—while mor-

tality within five years after ulcer diagnosis ranges from 30% to 40%, exceeding the survival rate of several types of cancer (ARMSTRONG; BOULTON; BUS, 2017).

PATHOPHYSIOLOGY OF IMPAIRED HEALING IN DIABETICS

The tissue healing process comprises four interdependent and overlapping phases: hemostasis, inflammation, proliferation, and remodeling (GUO; DI PIETRO, 2010). During hemostasis, a clot forms and platelet growth factors are released, initiating the reparative response. The inflammatory phase, mediated by neutrophils and macrophages, has as its main function the decontamination of the lesion and preparation of the bed for cell proliferation. In the proliferative phase, angiogenesis, granulation tissue formation, extracellular matrix deposition, and re-epithelialization are observed. Finally, remodeling involves the reorganization of collagen fibers and the progressive replacement of immature scar tissue with more resistant tissue (EMING; MARTIN; TOMIC-CANIC, 2014).

In diabetes mellitus, chronic hyperglycemia compromises all these stages through multiple mechanisms. Excessive formation of advanced glycation end products (AGEs) induces vascular stiffness and binds to their receptors (RAGE), perpetuating inflammation and oxidative stress, critical factors in the pathogenesis of diabetic foot ulcers (HUIJBERTS; SCHAPER; SCHALKWIJK, 2008). The inflammatory phase becomes prolonged, with inadequate macrophage recruitment and persistence of proinflammatory cytokines (TNF-α, IL-6). Angiogenesis is impaired by reduced VEGF expression and endothelial dysfunction. Additionally, collagen synthesis and maturation are deficient due to the inhibition of prolyl hydroxylase and increased activity of metalloproteinases (MMPs), resulting in fragile scar tissue prone to recurrence (BREM; TOMIC-CANIC, 2007).

Chronic Hyperglycemia and Protein Glycation

Chronic hyperglycemia, characteristic of diabetes mellitus, triggers pathogenic metabolic changes through four main pathways: activation of the polyol pathway, increased formation of advanced glycation end products (AGEs), activation of the hexosamine pathway, and activation of protein kinase C (PKC) (BROWNLEE, 2001). It is essential to note that the correct term is glycation (a non-enzymatic reaction between reducing sugars and proteins/lipids), and not "glycosylation" (a physiological enzymatic process mediated by glycosyltransferases). AGEs accumulate in tissues through irreversible covalent binding to structural proteins (such as collagen and elastin), inducing vascular stiffness, endothelial dysfunction, and activation of the RAGE (*Receptor for AGEs*) receptor, which perpetuates oxidative stress and chronic inflammation (SINGH et al., 2014). These mechanisms are directly associated with the development of microvascular complications (retinopathy, nephropathy, neuropathy) and macrovascular complications (coronary artery disease, stroke), as well as changes in the integrity of the skin barrier, with reduced epidermal hydration, dermal collagen atrophy, and increased susceptibility to traumatic injuries (YAMAGISHI et al., 2011).

Impaired Wound Healing in Diabetes Mellitus

Chronic hyperglycemia induces molecular, cellular, and microvascular changes that compromise wound healing in diabetic patients. Among the mechanisms involved, excessive formation of advanced glycation end products (AGEs) contributes significantly to reparative cell dysfunction and inadequate inflammatory response.

Repair Cell Dysfunction

Fibroblasts isolated from chronic diabetic wounds show reduced proliferation compared to those from uninjured tissue (LOOTS et al., 1999). *In vitro* studies demonstrate that direct exposure to AGEs inhibits the viability and function of dermal fibroblasts (DAI et al., 2019). Additionally, exposure of keratinocytes to soluble AGEs reduces their mobility and proliferation (ZHU et al., 2011), while cultivation on previously glycated type I collagen compromises cell adhesion and re-epithelialization (MORITA et al., 2005).

Microvascular Vasculopathy

Activation of the RAGE receptor by AGEs induces endothelial dysfunction in arterioles through the activation of NADPH oxidase and the generation of oxidative stress (GAO et al., 2008). In diabetic patients, altered expression of matrix metalloproteinases (MMPs) and their inhibitors contributes to inadequate tissue remodeling and impaired angiogenic response in wounds (LOBMANN et al., 2002).

Compromised Immune Response

The binding of AGEs to RAGE activates pro-inflammatory signaling pathways and amplifies the production of reactive oxygen species, damaging epithelial cells and compromising the function of neutrophils and macrophages (YAMAGISHI et al., 2012). This immune dysfunction favors bacterial colonization and chronic lesions (RAJA et al., 2023).

Clinical Implications

The triad of peripheral neuropathy, tissue ischemia, and infection constitutes the pathophysiological pillars of refractory diabetic foot ulcers (BOULTON et al., 2005). Therapeutic strategies with established evidence include strict glycemic control, adequate debridement, mechanical offloading, and judicious use of topical growth factors (BREM; TOMIC-CANIC, 2007). Although dietary interventions to reduce exogenous AGEs have theoretical potential (STIRBAN et al., 2015), their clinical efficacy in accelerating wound healing remains under investigation.

IN DIABETIC ENVIRONMENTS: MOLECULAR AND BIOCHEMICAL MECHANISMS

Immune homeostasis is profoundly compromised in the context of diabetes mellitus due to metabolic and oxidative changes that affect both innate and adaptive immunity. These dysfunctions contribute to chronic inflammation, recurrent infections, and defects in tissue repair (BERBUDI et al., 2020; ALEXANDER et al., 2024).

Innate Immunity Dysfunction, Molecular Mechanisms: Neutrophils: Compromised Chemotaxis and Phagocytosis

In diabetic environments, chronic hyperglycemia promotes the formation of reactive oxygen species (ROS) via NADPH oxidase activation and glucose auto-oxidation, generating oxidative stress that compromises multiple neutrophil functions (ALBA-LOUREIRO et al., 2007). This environment impairs neutrophil function through multiple mechanisms:

Impaired Chemotaxis

Neutrophils isolated from diabetic patients show reduced chemotactic migration capacity in response to inflammatory stimuli (DELAMAIRE et al., 1997). Studies show that exposure to glycemic concentrations in the diabetic range impairs chemotaxis signaling mediated by the formyl peptide receptor (FPR) (ROY et al., 2022). Additionally, activation of the AGEs-RAGE axis contributes to endothelial dysfunction and compromises the adequate extravasation of neutrophils to the site of injury (WANG et al., 2017).

Impaired Phagocytosis and Microbicidal Activity

The phagocytic capacity of neutrophils is significantly reduced in patients with type 2 diabetes mellitus, correlating inversely with glycated hemoglobin (HbA1c) levels (LECUBE et al., 2011). The production of ROS by NADPH oxidase is altered in diabetic neutrophils, with an increased initial response but inadequate sustainment during phagocytosis, compromising the effective destruction of pathogens (ALBA-LOUREIRO et al., 2007). Pharmacological blockade of RAGE signaling has been shown to partially improve the phagocytic function of neutrophils in experimental models of diabetic wounds (WANG et al., 2017).

Altered Apoptosis

The regulation of neutrophil apoptosis in diabetes presents complex behavior: while some studies demonstrate delayed spontaneous apoptosis in vitro (ALBA-LOUREIRO et al., 2007), others indicate that diabetic neutrophils have reduced viability and increased DNA fragmentation under conditions of metabolic stress. This dysfunction in cell survival regulation contributes to chronic inflammation and the inability to adequately resolve the inflammatory response in diabetic wounds.

Macrophages: Imbalanced Polarization and Chronic Inflammation

The diabetic microenvironment induces changes in macrophage polarization in wounds, with a predominance of the pro-inflammatory M1 phenotype over the reparative M2 phenotype, contributing to chronic inflammation and healing defects (MIRZA; KOH, 2011).

Activation of Pro-inflammatory Pathways

The binding of advanced glycation end products (AGEs) to the RAGE receptor activates the NF-κB pathway in immune system cells, increasing the expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and inducible nitric oxide synthase (iNOS) (YAMAGISHI et al., 2012).

Metabolic Reprogramming

Macrophages polarized to the M1 phenotype have a predominantly glycolytic metabolism, with increased glucose uptake and lactate production even in the presence of oxygen (Warburg effect). In contrast, M2 macrophages depend on mitochondrial oxidation of fatty acids (β-oxidation) and the Krebs cycle for energy generation (VIOLA et al., 2019).

Consequences for Healing

The persistent accumulation of M1 macrophages in diabetic wounds perpetuates a chronic inflammatory state, with continuous release of proteases and reactive oxygen species that damage the extracellular matrix and prevent the transition to the proliferative phase of healing (MIRZA; KOH, 2011).

Mitochondrial Dysfunction in T Lymphocytes in Diabetes Mellitus

Mitochondria are targeted by metabolic toxicity in diabetes, directly compromising T cell function. In patients with type 1 diabetes, T cells exhibit mitochondrial membrane hyperpolarization and accumulation of reactive oxygen species, associated with reduced proliferation after stimulation (Chen et al., 2017). In type 2 diabetes, senescent subpopulations of CD8+ EMRA lymphocytes exhibit fragmented mitochondria, oxidative phosphorylation dysfunction, and alterations in nutrient status in the glucose-rich microenvironment (Callender et al., 2021). These mitochondrial alterations compromise the metabolic transition necessary for T cell effector activation—from oxidative phosphorylation to aerobic glycolysis—resulting in functional anergy. Clinically, this dysfunction is fundamental to the increased susceptibility to infections and suboptimal *vaccine* response in diabetic patients (Berbudi et al., 2020).

B Lymphocytes in Diabetes Mellitus

B lymphocyte function shows distinct alterations in the two main types of diabetes mellitus. In type 1 diabetes, autoreactive B lymphocytes contribute to pathogenesis by presenting pancreatic islet antigens (GAD65, IA-2) to T lymphocytes and producing specific autoantibodies, detectable in 70% to 80% of patients at diagnosis (HAWA et al., 2000). In addition, patients with type 1 diabetes exhibit impaired primary humoral immune response to new viral antigens, with significantly reduced antibody production after hepatitis A vaccination compared to healthy controls (EIBL et al., 2002).

In type 2 diabetes, B lymphocytes acquire a pro-inflammatory phenotype characterized by increased secretion of TNF-α and IL-6. These cytokines promote the activation of Th1 effector T lymphocytes and aggravate insulin resistance in peripheral tissues (DEFURIA et al., 2013). Clinically, patients with type 2 diabetes have an attenuated vaccine response, with significantly lower levels of anti-RBD IgG antibodies after immunization with inactivated SARS-CoV-2 vaccines (XIANG et al., 2023).

Additionally, circulating immunoglobulin G (IgG) undergoes non-enzymatic glycation in hyperglycemic environments. Studies show a significant increase in glycated IgG in the plasma of diabetic patients (DANZE et al., 1987), and *in vitro* experiments confirm that this modification com-

promises the ability of the Fc region to bind complement and interact with Fcγ receptors, reducing the effectiveness of bacterial opsonization (KENNEDY et al., 1994).

These changes in B lymphocyte function and antibody quality directly contribute to the increased susceptibility to bacterial infections and suboptimal vaccine response observed in diabetic patients. Future studies should explore interventions that specifically reverse these biochemical changes, aiming to restore immune homeostasis in diabetic patients.

MICROVASCULAR CHANGES IN DIABETES MELLITUS

Diabetic microangiopathy is a systemic microvascular complication characterized by structural and functional changes in small-caliber vessels (arterioles, capillaries, and venules), with significant clinical repercussions in the vascularized beds of the retina, kidney, and peripheral nervous system (FORBES; COOPER, 2013). Chronic hyperglycemia acts as a primary triggering factor, activating interconnected metabolic pathways that converge on endothelial dysfunction, exacerbated oxidative stress, and impaired tissue angiogenesis (BROWNLEE, 2005). This article reviews the molecular and biochemical mechanisms underlying diabetic endotheliopathy, based on verifiable scientific evidence.

ENDOTHELIOPATHY: MOLECULAR AND BIOCHEMICAL MECHANISMS

Endothelial dysfunction represents an early pathogenic event in diabetic microangiopathy, preceding structural clinical manifestations and characterized by reduced nitric oxide (NO) bioavailability and a disproportionate increase in reactive oxygen species (ROS) (GIACCO; BROWNLEE, 2010).

Decoupling of endothelial nitric oxide synthase (eNOS)

Hyperglycemia induces a reduction in the availability of the cofactor tetrahydrobiopterin (BH4) through peroxynitrite-mediated oxidation, resulting in enzymatic uncoupling of eNOS. In this pathological state, the enzyme transfers electrons to molecular oxygen instead of L-arginine, generating superoxide (O_2^-) instead of NO, creating a vicious cycle of oxidative stress (BAKKER et al., 2009).

Formation of advanced glycation end products (AGEs) and activation of the RAGE receptor

Non-enzymatic glycation of structural and functional proteins generates AGEs through Maillard reactions, whose accumulation is accelerated in a hyperglycemic environment (YAMAGISHI et al., 2012). AGEs interact with the RAGE receptor (receptor for advanced glycation end-products), activating the NF-κB signaling pathway by degradation of IκBα and subsequent nuclear translocation of p65 (BIERHAUS et al., 2001). This transcriptional activation increases the expression of proinflammatory cytokines (TNF-α, IL-6) and adhesion molecules (VCAM-1, ICAM-1), promoting leukocyte adhesion to the endothelial surface and perpetuating chronic inflammatory response (YAMAGISHI; MATSUI, 2010).

Mitochondrial oxidative stress

Excess glycide substrate increases electron flow through the mitochondrial transport chain, raising the inner membrane potential beyond the physiological threshold, which favors electron leakage in complexes I and III with the formation of mitochondrial superoxide (O_2^-) (NISHIKAWA et al., 2000). This superoxide neutralizes residual NO, forming peroxynitrite ($ONOO^-$), a potent nitrating agent that inactivates critical enzymes and damages mitochondrial DNA (mtDNA), perpetuating bioenergetic dysfunction in a self-perpetuating cycle (GIACCO; BROWNLEE, 2010). Normalization of mitochondrial superoxide production blocks three pathogenic pathways of hyperglycemia: PKC activation, AGE formation, and activation of hexosamine and polyol pathways (NISHIKAWA et al., 2000).

Diabetic microangiopathy results from the pathogenic integration of multiple metabolic pathways activated by chronic hyperglycemia, with endothelial dysfunction occupying a central position in the damaging cascade. The uncoupling of eNOS, AGEs/RAGE/NF-κB signaling, and mitochondrial oxidative stress constitute potential therapeutic axes whose modulation may delay early microvascular changes. Future research should prioritize multimodal approaches that simultaneously attenuate oxidative stress and preserve endothelial function.

CAPILLARY LOSS IN DIABETIC MICROANGIOPATHY: VASCULAR DEGENERATION AND ENDOTHELIAL APOPTOSIS

Capillary rarefaction is a structural change characteristic of diabetic microangiopathy in peripheral tissues, resulting from three interconnected mechanisms:

Endothelial apoptosis: Chronic hyperglycemia induces programmed cell death in endothelial cells and pericytes of retinal capillaries, with progressive loss of these cells observed in experimental models of diabetes (HAMMES et al., 2002).

Basement membrane remodeling: TGF-β1 overexpression in diabetic microvessels induces excessive deposition of type IV collagen and fibronectin, promoting basement membrane thickening by up to 2.5 times (Caramori et al., 2003; Forbes & Cooper, 2013). This structural change reduces oxygen/nutrient diffusion and prevents adaptive vascular remodeling, perpetuating chronic tissue ischemia (Brownlee, 2005).

Angiogenic dysfunction: Angiogenic dysfunction in diabetes involves a reduction in the number of circulating endothelial progenitor cells (EPCs), which express the markers CD34, CD133, and VEGFR2, as well as impaired proliferation, adhesion, and incorporation into vascular structures. Hyperglycemia and advanced glycation end products (AGEs) induce defective VEGF signaling, including inactivation of the FLK-1 receptor (VEGFR2), impairing bone marrow EPC recruitment and endothelial migration. These defects contribute to inadequate arteriogenesis in diabetic is-

chemia and to the high prevalence of diabetic foot ulcers, which precede 84% of diabetes-related lower limb amputations. The dysfunction is also associated with reduced nitric oxide bioavailability and excessive oxidative stress (Kolluru et al., 2012).

Impact on Angiogenesis and Tissue Repair

Angiogenesis is a fundamental stage in the healing process, responsible for the formation of granulation tissue and the restoration of the blood supply necessary for tissue repair. Under physiological conditions, this process is coordinated by a complex network of growth factors, signaling pathways, and cellular interactions that ensure the formation of mature and functional capillary networks (Eming et al., 2014).

Diabetes promotes a persistent proinflammatory state characterized by the predominant polarization of macrophages to the M1 phenotype, which secrete high concentrations of TNF-α, IL-1β, and IL-6. These cytokines not only perpetuate endothelial damage by inducing reactive oxygen species (ROS), but also directly suppress the expression of VEGF and other angiogenic factors (Mirza & Koh, 2023).

CLINICAL CONSEQUENCES

Impaired healing in diabetics results from delayed re-epithelialization due to keratinocyte dysfunction, abnormal collagen deposition with formation of fragile scar tissue, and increased susceptibility to infections due to local immunosuppression (Armstrong et al., 2017). Chronic hyperglycemia induces microangiopathy, oxidative stress, and persistent inflammatory response, creating a microenvironment that is adverse to tissue repair (Falanga, 2019). These changes significantly increase the risk of recurrent ulcers, bacterial infections, and complications that can progress to amputation (IWGDF, 2020).

ACTION OF INSULIN IN TISSUE REPAIR

Healing in diabetics is impaired mainly by chronic hyperglycemia, which induces oxidative stress, microangiopathy, and immune dysfunction (Brownlee, 2001). Insulin plays an indirect physiological role by restoring normoglycemia, allowing a microenvironment suitable for repair. Experimental studies show that insulin administered locally at a pharmacological dose accelerates re-epithelialization via Akt/ERK activation in keratinocytes and M2 macrophage polarization (Lima et al., 2012; Yu et al., 2019). However, this effect does not represent a basal physiological mechanism of healing, but rather an adjuvant therapeutic strategy for refractory diabetic ulcers.

Modulation of the Inflammatory Response

Hyperglycemia in diabetic patients perpetuates chronic inflammation through activation of the NF-κB pathway, resulting in increased production of proinflammatory cytokines, such as TNF-α and IL-6, which impair the transition to the proliferative phase of healing (Kumar et al., 2020). Insulin acts as an anti-inflammatory modulator by reducing NF-κB activity, decreasing the release of these cytokines, and promoting a microenvironment favorable to tissue repair (Santos et al., 2021). In addition, insulin stimulates the polarization of macrophages to the reparative M2 phenotype, mediated

by the activation of PPARγ, accelerating the resolution of inflammation and the regeneration of damaged tissue (Zhang et al., 2022). These mechanisms highlight the potential therapeutic role of insulin in modulating the inflammatory response in chronic wounds associated with diabetes.

Hyperglycemia prolongs the inflammatory phase in diabetic wounds through persistent activation of NF-κB and elevation of TNF-α and IL-6, impairing the transition to the proliferative phase (BROWNLEE, 2001). Insulin exerts an acute anti-inflammatory effect by inhibiting the nuclear translocation of NF-κB and increasing the expression of IκBα in mononuclear cells, reducing pro-inflammatory cytokines (DANDONA et al., 2001). In experimental models, local insulin accelerates inflammatory resolution and stimulates macrophage polarization to the reparative M2 phenotype, partially mediated by the PI3K/Akt/PPARγ pathway (YU et al., 2019). However, PPARγ-dependent M2 polarization in diabetic wounds is more robustly documented for pharmacological agonists of this receptor than for physiological insulin (MIRZA et al., 2015).

Local Insulin in Diabetic Wounds: Investigational Approach

Topical application of insulin to skin wounds increases keratinocyte migration, accelerates re-epithelialization, and intensifies the fibroblastic reaction. The migration and differentiation of

Insulin-induced keratinocytes are dependent on the insulin receptor, but also on EGFR; furthermore, this effect is mediated by the PI3K-Akt-Rac1 pathway (Liu et al 2018).

Topical insulin treatment on burned skin improves collagen deposition and maturation, as evidenced by increased hydroxyproline levels (Dhall S. et al, 2015).

Human keratinocytes express functional insulin receptors, as demonstrated by binding assays with ^{125}I-insulin (~80,000 receptors/cell) (VERRANDO; ORTONNE, 1985). In a murine model, local insulin (0.03 U) accelerated the healing of excisional wounds by activating these receptors, stimulating keratinocyte migration via PI3K/Akt/Rac1 without altering systemic blood glucose (LIU et al., 2009). However, this is preclinical evidence without validated clinical translation: the IWGDF 2023 guidelines do not recommend local insulin for diabetic foot ulcers due to the absence of large randomized trials with low risk of bias (CHEN et al., 2023).

Stimulation of Angiogenesis

Insulin indirectly stimulates angiogenesis by activating the PI3K/Akt pathway in keratinocytes, promoting post-transcriptional biosynthesis of VEGF-A without altering the mRNA levels of the factor (GOREN et al., 2009). In a murine model, local insulin increased tissue expression of VEGF and eNOS phosphorylation in the bone marrow, with consequent mobilization of endothelial progenitor cells to the wound bed (LIMA et al., 2012).

Metabolic Regulation and Protein Synthesis

Tissue healing is not just a mechanical event, but a highly energy-demanding process that depends on **insulin** as its main metabolic conductor. By activating the **mTOR** pathway, this hormone acts as an anabolic

signal that authorizes fibroblasts to initiate the translation of essential proteins, such as collagen and elastin (**Saxton & Sabatini, 2017**). This molecular coordination is mediated by an intracellular signaling cascade that integrates the nutritional status of the cell with its structural growth (**Saltiel & Kahn, 2001**). Without this activation, the protein synthesis machinery remains dormant, preventing the reconstruction of the extracellular matrix.

However, the production of these proteins requires a constant supply of energy.

In this scenario, insulin regulates **energy metabolism** by optimizing glucose uptake and glycolytic efficiency, ensuring that **ATP** levels are sufficient to sustain cell proliferation and wound contraction (**Vatankhah et al., 2017**). Under conditions of insulin resistance, a collapse occurs: the cell suffers an ATP deficit at the same time that the mTOR pathway is silenced, resulting in stagnant and chronic wounds. Thus, interventions aimed at restoring local insulin signaling not only normalize blood glucose levels but also restore the cell's biosynthetic and bioenergetic capacity necessary for full repair (**Hrynyk & Neufeld, 2014**).

RANDOMIZED CLINICAL TRIALS WITH TOPICAL INSULIN

Local insulin demonstrates solid biological plausibility in experimental models for accelerating diabetic wound healing, with preclinical studies validating its molecular mechanism of action. In a murine model, topical application restored Akt (Ser473) and ERK1/2 (Thr202/Tyr204) phosphorylation in the wound bed, promoting keratinocyte survival, cell migration, and macrophage polarization to the anti-inflammatory M2 phenotype, without changes in systemic blood glucose (YANG et al., 2020). These findings are reproducible and support the hypothesis of local action independent of systemic insulin resistance, according to a systematic review that compiled animal and clinical evidence (WANG; XU, 2020).

However, translation into clinical practice faces critical methodological limitations. The main randomized clinical trial often cited (LIMA et al., 2012) was the subject of *an Expression of Concern* issued by *PLoS ONE* in February 2024 due to irregularities in Western Blot images and molecular data, requiring extreme caution in interpreting its conclusions (PLOS ONE, 2024). Subsequent clinical studies with locally deposited insulin present small samples (<60 patients), heterogeneity in formulations (cream, saline solution), and focus on distinct populations (pressure ulcers, non-diabetic foot), constituting low-quality evidence according to GRADE criteria (REZVANI et al., 2009; WANG; XU, 2020).

Given this scenario, local insulin should be positioned as an **experimental** strategy with potential for rigorous translational research, not as an established therapeutic standard. Critical gaps include: definition of the ideal formulation for controlled release, dose-response curve in injured tissue, and evaluation of synergies with other therapeutic modalities (YANG et al., 2020). New randomized, multicenter clinical trials with *in situ* molecular monitoring and robust clinical outcomes are needed to validate whether this low-cost approach can be integrated into chronic wound treatment protocols in the future (WANG; XU, 2020).

META-ANALYSES AND SYSTEMATIC REVIEWS

Local insulin has been of scientific interest for decades as a potential adjunct in wound healing, but its therapeutic consolidation remains limited by a lack of robust evidence. Until 2017, only one systematic review and preliminary meta-analysis gathered data from eight randomized clinical trials (seven included in the quantitative synthesis), with marked heterogeneity in the populations studied (diabetics, non-diabetics, acute and chronic wounds) and in the methodologies employed. Among the outcomes evaluated, **only the reduction in wound area** showed a statistically significant result in favor of local insulin (WMD = -6.59; 95% CI: -9.70 to -3.48), while the healing rate did not differ between groups (WMD = 0.04; 95% CI: -1.38 to 1.46) and the time to complete healing was evaluated by a single study, making statistical synthesis unfeasible. The quality of evidence was classified as **low** according to GRADE criteria, due to the small overall sample size and lack of methodological standardization. Given these limitations, the authors concluded that it is "difficult to draw definitive conclusions about the topical use of insulin for wound healing," recommending larger randomized trials with standardized protocols and a focus on homogeneous populations. (KANNAN; SIVARAMAKRISHNAN, 2017).

Recently, Ramírez-García-Luna et al. (2023) conducted a systematic review with Bayesian network meta-analysis that included 23 randomized clinical trials (n = 1,240 patients) evaluating different modalities of local insulin administration, including topical application, periulcer subcutaneous injection, and insulin irrigation. The results demonstrated statistically significant clinical benefits, with a 27% reduction in wound area, accelerated healing rate (23 mm^2/day), an average decrease of 10 days in the time to complete closure, and an odds ratio of 20 for complete healing with insulin versus control. Positive histological effects were also observed, including a 30 vessel/mm^2 increase in neoangiogenesis and a 25% increase in granulation tissue. It is important to highlight the favorable safety profile, with minimal change in blood glucose (-1.8 mg/dL) and no significant adverse events reported in the studies analyzed. However, the heterogeneity in the formulations evaluated and the absence of specific subgroup analysis for diabetic wounds with standardized glycemic control indicate that, although promising, the evidence still requires larger clinical trials and more standardized protocols before its routine adoption in specific populations (RAMÍREZ-GARCÍALUNA et al., 2023).

In one analysis, Bhuiyan, Adebayo, and Ahmed (2023) conducted a rigorous systematic review and meta-analysis evaluating the efficacy and safety of localized administration of insulin in wound healing in non-diabetic adults, including seven randomized clinical trials with a total of 735 participants. The analysis showed a significant improvement in the healing rate in the insulin-treated group (mean difference: 11.84 mm^2/day; 95% CI: 0.64–23.04; p = 0.04), as well as a reduction in wound area. However, the time to complete healing did not show a statistically significant difference between the groups (mean difference: -5.40 days; 95% CI: -11.28 to 0.48; p = 0.07). It is important to highlight the absence of adverse events related to the intervention, inclu-

ding hypoglycemia, hypokalemia, or local reactions in all studies analyzed. The authors acknowledged important limitations, such as small sample size in the primary studies, short follow-up duration in some trials, and methodological heterogeneity (I^2 = 97% for healing rate), concluding that, although localized insulin has shown benefits in accelerating healing in non-diabetics, larger prospective trials with standardized protocols and subgroup analysis by wound type and severity are needed to support definitive clinical recommendations (BHUIYAN; ADEBAYO; AHMED, 2023).

METHODOLOGICAL NOTE: Bhuiyan et al. (2023) evaluated only non-diabetic adults (n=735). Their results should not be extrapolated to diabetic patients, whose wound pathophysiology includes neuropathy, microangiopathy, and chronic immune dysfunction not present in this population. Although previous reviews have included diabetic patients in their analyses, such as the meta-analysis by Sridharan and Sivaramakrishnan (2017), cited by the authors themselves, no systematic review to date has focused exclusively on this population with standardized glycemic control protocols. This critical gap limits the direct comparability of findings and the clinical applicability of current evidence for wound management in diabetic individuals.

Note for Discussion

*The three syntheses converge on the safety of local insulin administration (absence of hypoglycemia), but diverge on the magnitude of clinical benefits and methodological rigor. The only meta-analysis with explicit GRADE assessment (Kannan 2017) classified the evidence as **low quality**, while the most recent syntheses (2023), although with greater sample power, maintain unresolved structural limitations, especially heterogeneity in formulations and the absence of robust analyses of diabetic patients with standardized glycemic control. These gaps justify caution in direct clinical extrapolation, despite the promising therapeutic potential.*

DISCUSSION

This review demonstrates that locally applied insulin—a term that encompasses topical formulations (creams, hydrogels) and periulcer subcutaneous administration—exerts plausible biological effects on wound healing, with well-characterized mechanisms in experimental models. *In vitro* and animal studies show that insulin activates the PI3K/Akt and MAPK/ERK signaling pathways, promoting keratinocyte migration, fibroblast proliferation, VEGF-mediated angiogenesis, and type I collagen deposition (LIU et al., 2009; LIMA et al., 2012). In diabetic wounds, where insulin-sensitive signaling is compromised by chronic hyperglycemia, local administration can partially restore these cellular processes essential for tissue repair (WANG; XU, 2020).

However, extrapolating these mechanisms to consolidated clinical benefit in humans requires methodological caution. The available quantitative syntheses reveal unresolved structural limitations. The pioneering meta-analysis by Kannan and Sivaramakrishnan (2017), although prospectively registered (PROSPERO CRD42016042183) and with explicit GRADE assessment, classified the evidence as **low quality** (⊕⊕⊖⊖) and identified significant benefit only in reducing wound area—with no significant difference in healing rate, granulation tissue, or microvessel

Comparative Table of Meta-analyses on Insulin in Wound Healing

Characteristic	Kannan & Sivaramakrishnan (2017)	Ramírez-GarcíaLuna et al. (2023)	Bhuiyan et al. (2023)
Population	Mixed (diabetics and non-diabetics)	Mixed (did not discriminate subgroups)	Exclusively non-diabetics
Sample	8 RCTs / n = 127	23 RCTs / n = 1,240	7 RCTs / n = 735
Primary significant outcome	Reduction in wound area ($p < 0.001$) Other outcomes: not significant	27% reduction in wound area Accelerated healing (23 mm^2/day) OR = 20 for complete healing	Increased healing rate ($p = 0.04$) Time to closure: not significant ($p = 0.07$)
Safety	No hypoglycemia/ adverse events	Minimal change in blood glucose (-1.8 mg/dL) No adverse events	Absence of hypoglycemia, hypokalemia, or local reactions
Quality of evidence	Small sample size, high heterogeneity	Not formally assessed (GRADE) Heterogeneity in formulations ("local insulin" includes subcutaneous injections)	High heterogeneity ($I^2 = 97\%$) Small sample size in primary studies
Conclusion by the authors	**Cautious**: *"difficult to draw definitive conclusions"*	**Optimistic**: *"promotes healing without significant adverse events"*	**Balanced:** benefit observed, but larger trials needed before clinical recommendations

density. The authors concluded that it was "difficult to draw definitive conclusions" due to the small sample size (n = 127) and methodological heterogeneity (KANNAN; SIVARAMAKRISHNAN, 2017).

Ramírez-GarcíaLuna et al. (2023) substantially increased statistical power (23 trials, n = 1,240 patients) and reported multiple positive outcomes, including a 27% reduction in wound area and an odds ratio of 20 for complete healing. However, the synthesis evaluated "local insulin"—a broad concept that includes subcutaneous injections and irrigation—and did not perform a rigorous subgroup analysis for diabetic wounds with standardized glycemic control, limiting the specific clinical applicability for this population (RAMÍREZ-GARCÍALUNA et al., 2023). In turn, Bhuiyan et al. (2023) conducted the only meta-analysis focused exclusively on non-diabetics (n = 735), demonstrating an increase in the healing rate (WMD = 11.84 mm^2/day; p = 0.04), but no significant difference in the time to complete closure (p = 0.07). This population specificity, although methodologically advantageous in avoiding metabolic confounding, does not directly support claims about efficacy in diabetic patients, the clinical population most relevant to the topic in question (BHUIYAN; ADEBAYO; AHMED, 2023).

The critical gap not filled by any of the available syntheses is the absence of a robust meta-analysis, with a registered protocol and GRADE assessment, that exclusively evaluates diabetic wounds with strict monitoring of systemic glycemic control. This limitation is fundamental, as healing dysfunction in diabetes involves multifac-

torial mechanisms (neuropathy, vasculopathy, altered inflammatory response) that are not fully replicated in non-diabetic models or in mixed populations without adequate stratification.

From a practical standpoint, human insulin has indisputable advantages in terms of accessibility and safety: it is a generic drug, widely available in the Brazilian Unified Health System (SUS), with a cost significantly lower than recombinant growth factors (US$ 1,500–10,000/mg) or advanced therapies (LIMA et al., 2012). All clinical studies included in the meta-analyses reported no hypoglycemia or significant adverse events with local administration, which is relevant for safety in outpatient or home settings. However, accessibility does not replace methodological requirements: the incorporation of any intervention into clinical protocols requires robust evidence of efficacy, not just logistical availability.

Despite the robustness of preclinical findings and promising results from clinical trials, the literature still lacks multicenter studies with large samples, standardized outcomes (e.g., time to complete closure, recurrence at 12 weeks), and direct comparison with local gold standards (e.g., silver sulfadiazine, hydrocolloids). In addition, few studies have explored optimized formulations for sustained release or stability in exudative wounds. Future research should prioritize clinical protocols adapted to the reality of the SUS, with monitoring of functional outcomes and cost-effectiveness analysis. Until such evidence is produced, local insulin can be considered a **promising experimental therapeutic option**, but not an established gold standard for the management of diabetic wounds.

CONCLUSION

Locally administered insulin in the wound bed demonstrates solid biological plausibility in experimental models, with well-characterized molecular mechanisms that include activation of the PI3K/Akt and ERK pathways, modulation of the inflammatory response by polarization of macrophages to the M2 phenotype, stimulation of VEGF-mediated angiogenesis, and promotion of keratinocyte migration. These effects occur independently of systemic blood glucose, conferring a favorable safety profile with no hypoglycemia in all clinical studies analyzed.

Although accessibility and low cost (generic drug available in the SUS) are relevant logistical advantages, they do not replace the methodological requirement for robust evidence before incorporation into clinical protocols. Multicenter randomized trials with rigorous monitoring of HbA1c, standardized clinical outcomes (time to complete healing, recurrence at 12 weeks), and comparison with current gold standards are necessary to define its specific therapeutic role in the management of diabetic wounds.

Together, these animal and clinical studies support the idea that locally administered insulin via drip improves wound healing through various mechanisms without causing side effects. Therefore, local insulin has shown promise in the field of wound healing, and further studies are needed to deepen our understanding of the role of insulin in the healing of different types of wounds.

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