

The Anti-inflammatory and Proangiogenic Properties of High-Density Lipoproteins: An Emerging Role in Diabetic Wound Healing

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Significance: Prolonged inflammation and impaired angiogenesis are the two principal factors that prevent successful wound healing, which is exacerbated in people with diabetes. There is a significant need for new wound healing treatments that target both these factors simultaneously. This review discusses the emerging evidence that high-density lipoproteins (HDL) have pleiotropic wound healing benefits.

Recent Advances: Numerous *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory and proangiogenic effects of HDL. In endothelial cells, HDL mediate these effects through interaction with the scavenger receptor SR-BI, which activates the PI3K/Akt pathway, causing a decrease in inflammatory protein production and an increase in proangiogenic growth factors. In macrophages, HDL inhibit inflammation through suppression of the nuclear factor kappa B activation pathway. This review details the molecular disturbances that cause impaired wound healing in diabetes with a particular focus on inflammation and angiogenesis and the pathways in which HDL provide benefit.

Critical Issues: Diabetic foot ulcers (DFUs) impose a major public health challenge worldwide. It is estimated that 20% patients with DFUs require amputation, which is accompanied by a significant social and economic burden. To date, there are no therapeutic agents with pleiotropic effects that actively improve wound healing, highlighting a therapeutic void for this complex disease.

Keywords: high-density lipoproteins, diabetes, anti-inflammatory, angiogenesis, wounds, ulcers

SCOPE AND SIGNIFICANCE

DIABETES IS ONE of the most common metabolic disorders,^{1,2} with the World Health Organization reporting 382 million diabetic people in 2013, with 592 million predicted by 2035.¹ Patients with diabetes have a predisposition for the development of peripheral arterial disease and peripheral neuropathy, both of which

are precursors for the development of diabetic foot ulcers (DFUs).^{3,4} It is estimated that 20% of patients with DFUs require amputation.³ The risk of death in patients with an existing DFU is 2.5 times higher than diabetic patients without.^{3,5} Wound healing is a complex physiological process and this process is complicated further by diabetes.⁶

TRANSLATIONAL RELEVANCE

There are currently very few therapies that actively improve wound healing and exhibit pleiotropic effects.⁷ Emerging evidence suggests high-density lipoproteins (HDL) possess significant wound healing properties through regulation of multiple important wound healing mechanisms. HDL have traditionally been viewed as a cardio-protective protein since the discovery of a significant inverse relationship between plasma HDL cholesterol levels and the risk of myocardial infarction.⁸ More recently, HDL have been reported to exhibit anti-inflammatory, endothelial protective, and proangiogenic effects in response to ischemia.^{9,10} As a result of these multiple benefits, HDL promise to be a highly effective wound healing agent.

CLINICAL RELEVANCE

This article will discuss the molecular mechanisms involved in diabetic wound healing, focusing on the inflammatory disturbances and impaired angiogenesis. We will compare the successes and limitations of current clinical strategies being tested to improve healing. Finally, this article will highlight the accumulating evidence supporting the role of HDL in diabetic wound healing, and why it may have advantages over other therapeutic approaches that will facilitate its translation into the clinical setting.

BACKGROUND

Acute and chronic wounds

Wound healing is a dynamic process consisting of four continuous, yet overlapping, phases characterized by hemostasis, inflammation, proliferation, and remodeling (Fig. 1).¹¹ During optimal wound healing, these phases occur in a precise and timely manner.¹²

Some flexibility exists in the wound healing process to support imbalances within each step and sequence; however, if this not carefully regulated and system is disrupted too much, it can lead to the development of a chronic nonhealing wound. Diabetes causes substantial imbalances in each stage of the wound healing process, and if a wound fails to heal within the first 8 weeks in a diabetic patient, this is classified as “chronic.”⁶

Diabetes is a condition characterized by chronically elevated blood glucose levels. In many people with diabetes, this can be controlled with insulin therapy and/or other antidiabetic drugs. Despite this, the risk of impaired wound healing is significantly elevated in people living with diabetes and is associated with persistent, nonresolving inflammation and defective tissue repair responses.^{13,14} For example, in hyperglycemia, neutrophils produce more superoxide and proinflammatory cytokines.¹⁵ Neutrophils from diabetic patients are also found to be in a hyperactive state, indicated by increased

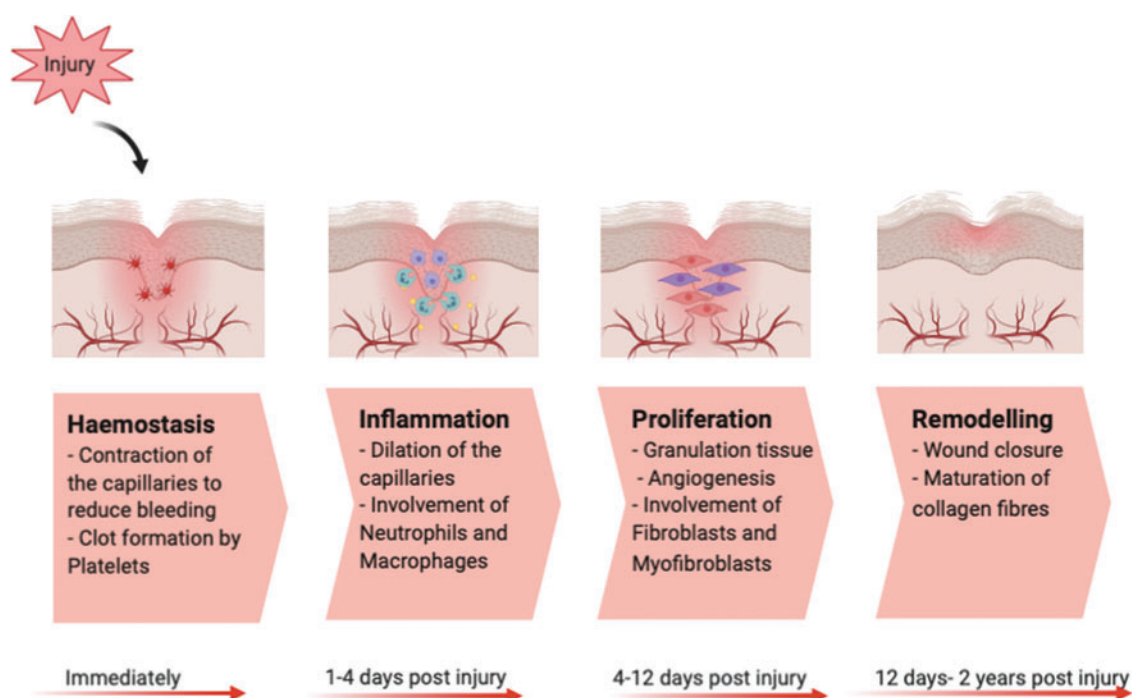


Figure 1. Different Stages of healthy wound healing in humans consisting of hemostasis, inflammation, proliferation, and remodeling, which usually occur immediately, 1–2 days, 4–12 days, and 12 days-2 years postinjury, respectively. Color images are available online.

expression of activation marker CD11b.¹⁶ In terms of the adaptive immune system, the study by Moura *et al.* reported decreased diversity of blood T cell T-cell receptor- β in diabetes, particularly in DFUs, despite the accumulation of T cells in the wound tissues of diabetic patients.¹⁷

Diabetes also increases the levels of circulating inflammatory protein C-reactive protein in foot wounds¹⁸ and wounds have increased total numbers of inflammatory cells, paralleled by decreased concentrations of growth factors.¹⁹ This hinders the migration of the epidermis layer over the wound, which is essential for healing.¹⁹ Diabetes also causes alterations in wound structure and the remodeling process, causing a loss of heparan sulfate proteoglycans such as syndecan-4²⁰ and increased degradation of the extracellular matrix.²¹ In addition, diabetes significantly impacts angiogenesis, which is the formation of new vessels from existing ones. Diabetic wounds have poor angiogenic responses resulting in decreased capillary density. This is due to the negative effect of hyperglycemia on different range of transcription factors, signaling molecules, and growth factors such as reactive oxygen species (ROS) and vascular endothelial growth factor (VEGF).

Limitations of current and previous topical wound healing therapies

Based on the evidence-based guidelines published by the International Working Group on Diabetic Foot (IWGDF), the most common treatment options for DFUs are limited to debridement of hypertrophic tissue surrounding the wound, application of appropriate dressings, and orthotic devices to offload the pressure from the ulcer area.²² In some cases, vascular reconstruction is required if macrovascular ischemia is contributing to chronic wound development.²²

A recent comprehensive review, part of the IWGDF process, identified that sharp debridement was the only intervention studied in a controlled manner and with adequate evidence demonstrating benefit.²³ For topical interventions, a sucrose octasulfate dressing was found to be the most effective in a recent well-controlled randomized multicenter clinical trial across France, Spain, Italy, Germany, and United Kingdom.²⁴ The mechanism of action is believed to be through inhibition of matrix metalloproteases, which are found in higher concentrations in chronic wounds, increasing inflammation and breaking down the remodeling process.²⁴

Negative pressure wound therapy (NPWT) (only when used after surgery), has demonstrated prom-

ising benefits in clinical trials.²⁵ However, for chronic wounds, there is not enough evidence to establish whether NPWT has any benefit over standard care. NPWT is predominantly recommended for acute and larger postsurgical wounds, which have excess exudate that impacts wound healing.

Growth factors are essential for wound healing, particularly for promoting angiogenesis.²³ Numerous clinical trials have tested the application of growth factors, including fibroblast growth factor (FGF), epidermal growth factor (EGF), combined FGF-EGF, and other growth factors, on diabetic wounds. Topical application of basal FGF in a spray format onto DFUs caused a 75% reduction in ulcer area; however, the analysis for this study was as per protocol analysis leading to bias.²⁶ Furthermore, a recent Phase III multicenter clinical trial used a spray form of recombinant human EGF that significantly improved wound healing in 167 patients with DFUs.²⁷ Despite their promise, a major confounder for the translation of growth factors is their lack of cost-effectiveness, with an additional significant consideration being that they only target a single mechanistic pathway of healing.

In summary, despite numerous clinical trials that have tested a wide variety of interventions, there is still no universally approved or effective therapy for diabetic wound healing, which has been implemented to support the best standard of care. Most clinical interventions target only a single mechanism of action in the wound healing process. This may be insufficient to effectively heal mechanistically complex diabetic wounds. Agents that offer pleiotropic actions on the wound healing process are therefore far more likely to be effective.

DISCUSSION

HDL: the "Good Cholesterol"

HDL is primarily composed of an outer layer of phospholipids and proteins (known as apolipoproteins, predominantly apoA-I) and a hydrophobic core of cholesterol (esterified and nonesterified) and triglycerides.²⁸ Circulating plasma HDL comprise a highly heterogeneous family of lipoprotein particles varying in density, size, surface charge, and lipid/protein composition.²⁹ HDL interact with three key cell surface proteins. These are the cholesterol transporters ABCA1 and ABCG1 and the scavenger receptor SR-BI.³⁰ A vast number of *in vitro*, *in vivo*, and epidemiological studies have demonstrated that HDL exhibit atheroprotective, antithrombotic, antioxidant, antiapoptotic, anti-inflammatory, antiproteolytic, and proangiogenic properties.²⁹ HDL are characterized into several

subtypes based on variations in size and composition; these subtypes include HDL₂ and HDL₃.²⁸ HDL₃ in particular is believed to be a subpopulation of HDL with superior anti-inflammatory effects.³¹ The majority of the beneficial effects of HDL are conducive to improving wound repair.

Epidemiological evidence of a role for HDL in wound healing

A large body of evidence supports a relationship between low circulating HDL cholesterol levels and the risk of a cardiovascular event.³² More recently, epidemiological studies have shown an inverse relationship between HDL levels and the risk of developing diabetes. For instance, levels of HDL₂ were found to be inversely proportional with the incidence of type 2 diabetes.³³ Interestingly, lower levels of HDL have been associated with an increased risk of developing diabetic neuropathy, an important contributing factor for DFU formation, in both type 1 and type 2 diabetes.³⁴ While pharmacological elevation of circulating HDL cholesterol levels using cholesterol ester transfer protein (CETP) inhibitors was not effective in reducing cardiovascular events, interestingly, it did delay the onset of T2DM, indicating an antidiabetic effect.³³

Only one clinical study has specifically evaluated the relationship between endogenous HDL cholesterol levels and diabetic wound healing outcome.³⁵ In 163 patients with an existing DFU in a single hospital in Japan, this study found that there was an inverse correlation between circulating HDL cholesterol levels and three endpoints: (1) minor amputation, or below the ankle amputation, (2) major amputation or amputation above the ankle, and (3) wound-related death, defined as a death with unhealed ulcer. This independently suggested that endogenous HDL cholesterol is a predictor for lower extremity amputation.

Inflammation in diabetic wound healing and the role of HDL

Inflammation is important in the early stages of wound healing to combat infection, however, when prolonged and inappropriate inflammation negatively affect wound healing at every step in the repair process. Diabetes, in particular, exacerbates inflammation and substantially slows its resolution, causing multiple complications in wound biology and healing. Agents that suppress inflammation are therefore likely to have significant wound healing benefits. It is well established that HDL have anti-inflammatory effects that include (1) the suppression of endothelial cell inflammation and improved endothelial function and (2) the reduction of monocyte/macrophage activation and

recruitment to sites of inflammation (Table 1).²⁸ These properties of HDL all have benefits on the wound healing process and the prevention of chronic wound development.

Endothelial protective effects. Endothelial cells play a key role in wound healing. They are the building blocks of wound neovessels that are essential for repair. Through their expression of adhesion molecules, chemokines, and cytokines, endothelial cells mediate the delivery of monocytes and macrophages to the wound site. Controlled endothelial cell inflammation is therefore critical for successful healing, but can lead to chronic wound development if inappropriately prolonged or elevated. HDL exhibit potent inhibitory effects on endothelial cell inflammation (Fig. 2). For example, HDL have been shown to reduce the *in vitro* and *in vivo* expression of key adhesion molecules, including intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin.^{31,36} One of the mechanisms for the anti-inflammatory effects of HDL on endothelial cells starts with the interaction between HDL and the sphingosine-1-phosphate 3 (S1P3) receptor. This results in the phosphorylation and activation of the PI3K/Akt pathway, which leads to the activation of endothelial nitric oxide synthase (eNOS) and the production of nitric oxide (NO). While NO plays an important role in the promotion of wound angiogenesis and maintenance of endothelial function, it also exhibits anti-inflammatory effects. It has been demonstrated that NO can inhibit the tumor necrosis factor (TNF)- α activation pathway through the suppression of nuclear factor kappa B (NF- κ B); the pivotal inflammatory transcription factor comprised subunits p65 and p50 that normally promotes VCAM-1, ICAM-1, and chemokine expression.³¹ Reconstituted HDL (rHDL, apoA-I complexed with phospholipid) also suppress endothelial cell che-

Table 1. Anti-inflammatory effects of high-density lipoproteins

Study	Mechanism of Action for HDL's Anti-inflammatory Properties
Kimura <i>et al.</i> ³⁶	Inhibition of key cell adhesion molecules, including VCAM-1 and ICAM-1
Yuhanna <i>et al.</i> ⁶⁴	Stimulating eNOS and production of nitric oxide
Bursill <i>et al.</i> ³⁷	Suppressing endothelial cell chemokine expression (CCL2, CCL5, and CX ₃ CL1)
Murphy <i>et al.</i> ¹⁰	Decreasing the expression of monocyte CD11b in a dose-dependent manner
Sanson <i>et al.</i> ⁴³	Promoting the transition of M1-like macrophages to M2-like macrophages
Liu <i>et al.</i> ⁴⁴	Inhibition of inflammatory cytokine production from monocytes

eNOS, endothelial nitric oxide synthase; HDL, high-density lipoproteins.

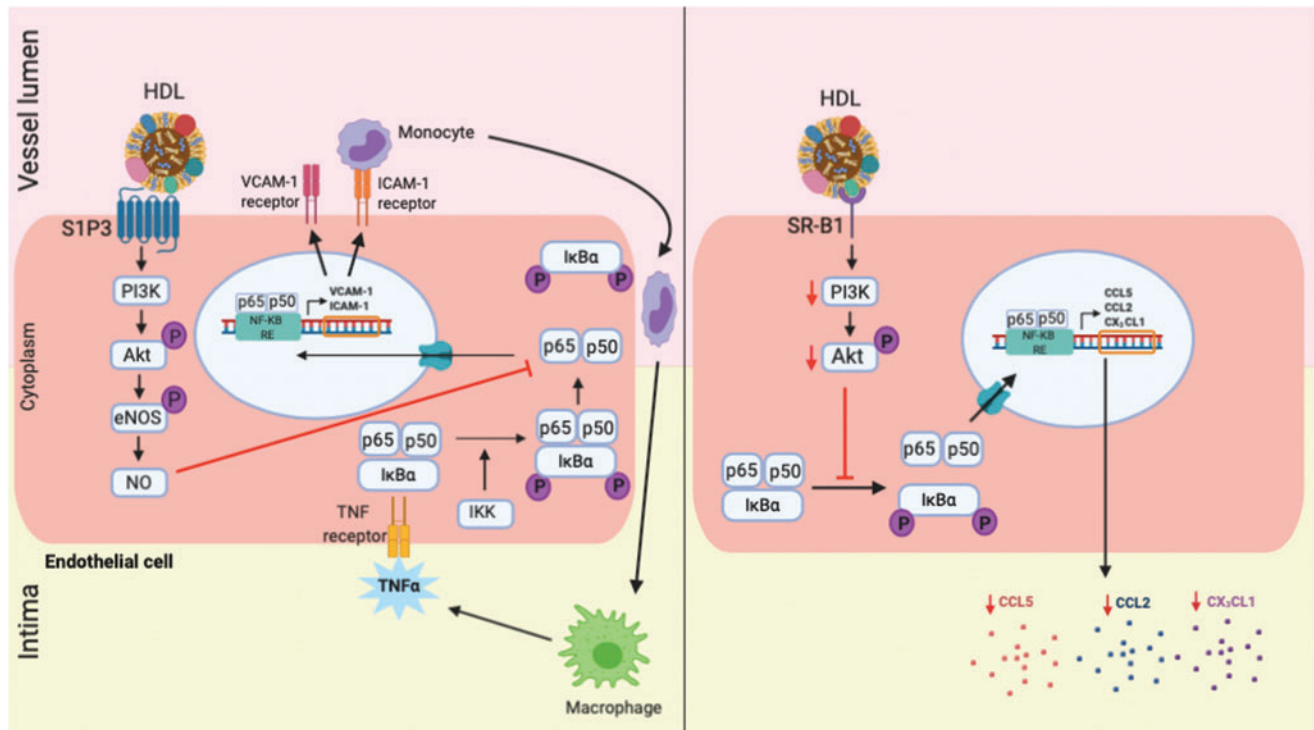


Figure 2. Mechanisms for the anti-inflammatory effects of HDL in endothelial cells. *Left panel:* HDL increase formation of bioactive nitric oxide (NO) through interaction with the S1P3 receptor, which activates PI3K/Akt, leading to inhibition in action of NF- κ B. This prevents the translocation of the NF- κ B subunits p65/p50 to the nucleus, where it binds to the NF- κ B response elements (RE) in the promoter region of inflammatory genes, including vascular cell adhesion molecule (VCAM)-1 and intercellular cell adhesion molecule (ICAM)-1. *Right panel:* in an alternative pathway, HDL reduce the production of inflammatory cytokines CCL2, CCL5, and CX₃CL1 through interaction with scavenger receptor (SR)-B1 that leads to suppression of downstream kinase signaling (PI3K/Akt) and inhibition in the activation of NF- κ B pathway. NF- κ B, nuclear factor kappa B; S1P3, sphingosine-1-phosphate 3. Color images are available online.

mokine expression, including CCL2, CCL5, and CX₃CL1. This is mediated through interaction with SR-B1, inhibition of downstream kinase signaling, and suppression of NF- κ B activation.³⁷

Preclinical models of vascular inflammation have also demonstrated the anti-inflammatory effects of HDL. For example, in a rabbit model of carotid occlusion,³⁸ systemic infusion of rHDL inhibited neutrophil infiltration and the expression of VCAM-1, ICAM-1, and the chemokine CCL2.³⁸ HDL have also been reported to increase the anti-inflammatory enzyme platelet-activating factor acetyl hydrolase, which inactivates platelet-activating factor and promotes inflammation.³⁹

Macrophages. Macrophages are an important cell type contributing to both the initiation and resolution of the inflammatory phase of wound healing.⁴⁰ Macrophages are highly heterogeneous and can express a wide range of markers with an adaptable and plastic nature.⁴¹ The two prominent macrophage populations that are active at the wound site during the wound healing process are tissue-resident macrophages and monocyte-derived macrophages. Under normal uninjured conditions,

the population of tissue-resident macrophages is higher than monocyte-derived macrophages. However, upon injury, an influx of monocyte-derived macrophages results in a twofold increase in their number and an acute reduction in tissue-resident macrophages.⁴⁰ Shortly after injury and hemostasis, monocyte-derived macrophages enter the wound site and differentiate into proinflammatory (M1) macrophages releasing inflammatory cytokines, including interleukin (IL)-1 β , TNF- α , and IL-6.⁴² This is followed by the subsequent healing stages through polarization into the anti-inflammatory (M2) reparative macrophages, which release anti-inflammatory cytokines such as transforming growth factor (TGF)- β and IL-10 and support tissue remodeling and fibrosis.⁴² In pathological conditions such as diabetes, the transition from the M1 to the M2 phenotype is delayed, causing an increase in wound inflammation through excess M1-derived chemokine/cytokine expression. This persistent inflammation is likely to result in the development of a chronic wound.⁴⁰

Multiple studies have demonstrated that HDL have anti-inflammatory effects on human monocytes and macrophages, which is likely to have

substantial benefits on the wound healing process. For example, HDL prevent the activation of primary human monocytes by significantly decreasing the expression of CD11b.¹⁰ In an *in vitro* study,¹⁰ HDL inhibited phorbol 12-myristate-13-acetate (PMA)-induced activation of monocyte CD11b in a dose-dependent manner.¹⁰ Furthermore, when monocytes were pretreated with HDL followed by stimulation with PMA, there was a significant reduction in CD11b expression.¹⁰

HDL have also been shown to promote a shift from M1-like macrophages to M2-like macrophages. In a key study by Sanson *et al.*,⁴³ HDL suppressed both basal and interferon gamma-induced expression of M1 inflammatory markers such as inducible nitric oxide synthase, IL-6, and TNF- α . Furthermore, HDL caused a 10-fold increase in the expression of M2-like markers Arg-1 and Fizz-1.⁴³ It is important to note that both Arg-1 and Fizz-1 are likely to contribute to tissue remodeling in wound healing as they mediate the deposition of extracellular matrix in animal models.⁴⁴ These findings further highlight the biological plausibility of utilizing the anti-inflammatory properties of HDL for wound healing.

HDL also inhibit the production of inflammatory cytokines from monocytes. One mechanism by which HDL does this is through binding of the apoA-I component of HDL to the “stimulating factor” on circulating stimulated T lymphocytes. This blocks the normal interaction between monocytes and stimulated T lymphocytes, thereby preventing cytokine release (*e.g.*, IL-1 β and TNF- α) from monocytes at sites of inflammation (Fig. 3).⁴⁵ ApoA-I

has also been shown to inhibit the production of inflammatory cytokines such as IL-1 β , TNF- α , and intercellular ROS by blocking the interaction of T cell ligands with the leukocyte β 2-integrin subunit.³¹ Consistent with these findings, rHDL reduces the secretion of chemokines CCL2, CCL5, and CX₃CL1 from monocytes and the expression of chemokine receptors CCR2 and CX₃CR1.³⁷

HDL also suppress Toll-like receptor (TLR)-induced expression of proinflammatory cytokines from macrophages.⁴⁶ For example, pretreatment of bone marrow-derived macrophages with HDL reduced IL-6 and TNF- α secretion in response to a variety of TLR ligands. The mechanism for these effects of HDL was found to be through regulation of the transcription factor ATF3 that is downstream of the TLR. ATF3 is induced through TLR stimulation and acts through a negative feedback system to inhibit excessive production of proinflammatory cytokines. Interestingly, infusion of HDL into mice increased the level of ATF3 mRNA expression in the Kupffer cells (macrophages) of the liver. In the same study, HDL demonstrated an endothelial protective effect by increasing re-endothelialization of carotid arteries after carotid injury, an effect that was not observed in *Atf-3*-deficient mice.⁴⁶

Diabetes significantly prolongs wound inflammation and halts the normal sequence of the wound repair process. This includes disruption to wound macrophage regulation. In a study done by Mirza *et al.*,¹³ macrophages isolated from chronic wounds of patients with type 2 diabetes prominently exhibited a proinflammatory M1-like phe-

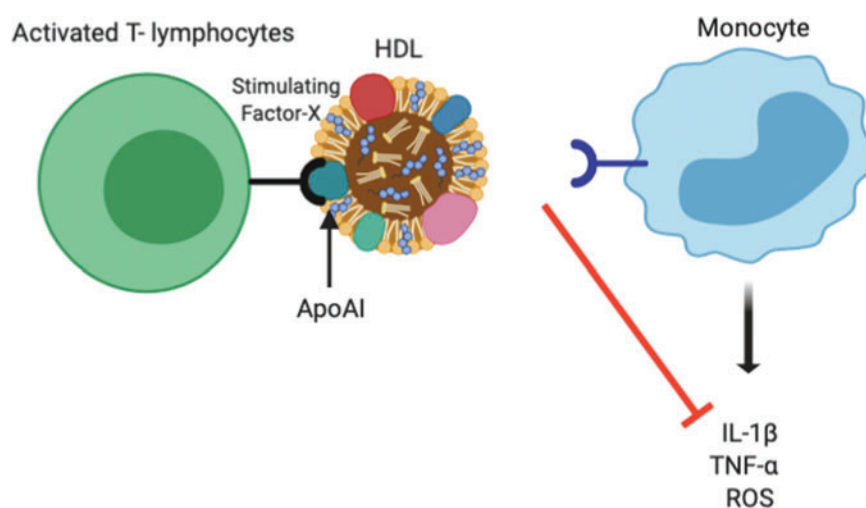


Figure 3. Anti-inflammatory effects of HDL on monocytes/macrophages. HDL block the interaction between stimulated T lymphocytes and monocytes by interacting with “stimulating factor X” on T lymphocytes. This reduces the interaction between stimulated T lymphocytes and monocytes, which in turn reduces monocyte secretion of inflammatory cytokines and ROS. ROS, reactive oxygen species. Color images are available online.

notype, expressing high levels of inflammatory cytokines, including IL-1 β , matrix metalloproteinase (MMP)-9, and TNF- α .¹³ Furthermore, wound macrophages obtained from diabetic mice persisted as proinflammatory M1-like macrophages and failed to transition to the reparative M2-like phenotype.¹³ The phenotypic switch from M1-like macrophages to M2-like macrophages is also believed to be controlled by factors such as IL-4/IL13, IL-1, TLR activation, and optimal activity of stress-associated MAPK, P38.⁴⁷ Suppression of inflammation can, however, overcome these issues. The use of an IL-1 β blocking antibody was found to improve wound healing, reduce M1-like macrophages, and increase the presence of M2-like macrophages in wounds.¹³ This highlights the importance of controlling inflammation to ensure successful wound healing in diabetes. Despite this, very few wound healing agents target inflammation. While HDL have potent anti-inflammatory properties, there is no published literature to date specifically reporting the anti-inflammatory effects of HDL in diabetic wounds. Our laboratory recently found that topical application of rHDL in a diabetic wound healing model was able to reduce the gene expression of inflammatory markers such as CCL2, IL-6, TGF- β , and *relA* in wound tissues, 3 days postwounding and treatment. This provides promising support for an anti-inflammatory effect of HDL in the context of diabetic wound healing.

Angiogenesis in wound healing and the role of HDL

One of the most important contributors to wound healing is angiogenesis.⁴⁸ Angiogenesis is the formation of new blood vessels from pre-existing vessels and is critical in the proliferation phase of wound healing.

Proangiogenic effects of HDL. A number of studies demonstrate a proangiogenic effect of HDL *in vitro* in response to hypoxia and in preclinical murine models of hind limb ischemia and wound healing (Table 2).⁴⁹ *In vitro*, HDL have been shown to significantly induce proliferation, migration, and tubule formation of endothelial cells, which are critical for angiogenesis. Investigation of the intracellular mechanisms has revealed that these changes in endothelial cell function occur through Src family kinases PI3K and MAPK with the involvement of SR-BI.⁵⁰ SR-BI has also been implicated as the receptor that mediates the augmentation of ischemia-driven angiogenesis in *in vivo* murine models of hindlimb ischemia and wound healing.⁵¹ This study delineated that HDL interaction with

Table 2. Proangiogenic effects of high-density lipoproteins

Study	Mechanism of Action for HDL's Proangiogenic Properties
Tan <i>et al.</i> ⁵² Cannizo <i>et al.</i> ⁵⁴ Primer <i>et al.</i> ⁵³ Tan <i>et al.</i> ⁵²	Inducing proliferation, migration, and tubule formation of endothelial cells
Cannizo <i>et al.</i> ⁵⁴	Augmenting VEGFR2 phosphorylation and downstream activation of critical signaling proteins involved in endothelial cell proliferation and migration
Tan <i>et al.</i> ⁵²	Stabilization of HIF-1 α , through increasing Siah 1/2 and decreasing PHD-1
Jin <i>et al.</i> ⁵⁵ Yuhanna <i>et al.</i> ⁶⁴	Activation of VEGFR2 through S1P3 signaling pathway Stimulating eNOS and production of nitric oxide

PHD, prolyl hydroxylase domain; S1P3, sphingosine-1-phosphate 3; VEGFR2, vascular endothelial growth factor receptor 2.

SR-B1 activates the PI3K/Akt pathway leading to increased levels of HIF-1 α and the transcription and production of VEGF-A (Fig. 4). Further investigation of the mechanisms of action revealed that in endothelial cells, rHDL is able to stabilize HIF-1 α in high glucose.⁵² It was found to do this through an increase in the ubiquitin ligases *Siah-1* and *Siah-2*, which subsequently suppress the prolyl hydroxylase domain (PHD) proteins PHD-2 and PHD-3 that normally target HIF-1 α for degradation. There is also emerging evidence that rHDL stabilizes HIF-1 α and improves angiogenesis in diabetes by correction of impaired metabolic reprogramming responses to hypoxia.⁵³

VEGF-A is a potent proangiogenic growth factor that is under the control of HIF-1 α . It activates numerous angiogenesis cellular activities once bound to its receptor, vascular endothelial growth factor receptor 2 (VEGFR2), on endothelial cells. Following binding, activation of the ERK1/2 and p38MAPK downstream signaling pathways occurs, which are important for cell proliferation and cell migration, respectively (Fig. 4). In a recent *in vitro* study, rHDL was shown to augment VEGFR2 phosphorylation and cause activation of downstream ERK1/2 and p38MAPK proteins in endothelial cells under hypoxic conditions, adding another piece to the puzzle of how rHDL promotes angiogenesis.⁵⁴

S1P is a bioactive lipid mediator that regulates angiogenesis, vascular stability, and permeability.⁵⁵ HDL transports 60% of the S1P in the plasma making it the main acceptor and carrier of S1P. It has been reported that HDL-mediated angiogenesis involves activation of VEGFR2 also through the S1P3 signaling pathway.⁵⁵ This occurs through binding of the HDL/S1P to S1P3 receptor, which then phosphorylates (activates) VEGFR2 and promotes angiogenesis functions, including proliferation, migration, and

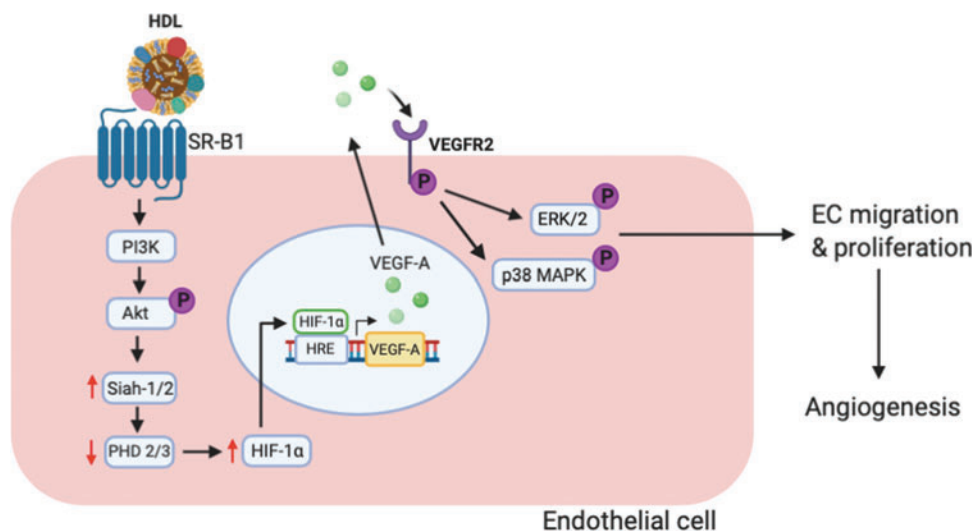


Figure 4. The proangiogenic effects of HDL in endothelial cells. Following interaction with scavenger receptor (SR)-B1, HDL activates downstream PI3K/Akt signaling. This increases HIF-1 α stabilization through an increase in the ubiquitin ligases Siah-1 and Siah-1, which inhibit the PHD proteins PHD-2 and PHD-4 that normally target HIF-1 α for degradation. With increased HIF-1 α stabilization, it allows for elevated levels of HIF-1 α translocation to the nucleus. In the nucleus, HIF-1 α binds to HIF response elements (HRE) in the promoter regions of proangiogenic genes, including VEGF-A, which increases their expression. This increases the interaction between VEGF-A and its receptor VEGFR2, leading to enhanced activation of ERK1/2 and p38 MAPK that promote endothelial cell migration and proliferation, respectively, and results in increased angiogenesis. PHD, prolyl hydroxylase domain; VEGFR2, vascular endothelial growth factor receptor 2. Color images are available online.

tubule formation in endothelial cells.⁵⁵ The Ras-activated MAP kinase pathway was the intracellular mechanism of action for these HDL-S1P/S1P3 receptor-driven proangiogenic effects of HDL.⁵⁶

Impaired angiogenesis and wound healing in diabetes. Diabetes leads to a significant impairment in angiogenesis as a result of a number of mechanisms. First, endothelial cells become dysfunctional when exposed to elevated systemic glucose concentrations, leading to a loss in their integrity and increased apoptosis.⁴ Second, high glucose causes imbalances in cytokine/chemokine and growth factor levels that disturb normal angiogenesis.

Studies have shown that the ability of VEGF-A to simulate the migration of monocytes from diabetic patients is impaired, when compared to monocytes from healthy individuals. This was despite higher levels of VEGF-A in the plasma of patients with diabetes indicating this is due to a deficiency in the activation of VEGFR2.⁵⁷ Furthermore, in diabetic mice wounds, the mRNA and protein levels of VEGF-A are significantly reduced, compared to healthy controls.⁵⁸ Higher levels of the antiangiogenic growth factor pigment epithelium-derived factor⁵⁹ and low levels of FGF-2 cell surface receptors syndecan-4 and glypican-1²⁰ in diabetic patients also lead to impaired angiogenesis in diabetes. Furthermore, increases in antiangiogenic molecules that cause proteolysis of

VEGF-A have been observed in chronic venous ulcers.^{60,61} Another vascular maturation pathway relevant to angiogenesis in diabetic wound is the Ang1/Ang2/Tie2 complex. In diabetic mice, the ratio of Ang1 to Ang2 has been decreased, which leads to reduced maturation of nascently formed blood vessels.⁴

Another reason for the impairment of angiogenesis in diabetes is that high glucose impairs angiogenesis responses to wound ischemia, a result of a low oxygen supply due to tissue injury or peripheral artery occlusion.⁴⁹ Central to this is the high-glucose-induced destabilization of HIF-1 α , the key transcription factor that is activated in response to hypoxia/ischemia to promote VEGF-A and angiogenesis.⁴⁹ Related to this is a reduction in the recruitment of endothelial progenitor cells (EPCs) to the site of diabetic wounds. EPCs make significant contributions to wound vascularization in response to ischemia. In diabetes, the production of the chemokine stromal cell-derived factor (SDF)-1 α in the wound is reduced. SDF-1 α normally facilitates the migration and recruitment of EPCs to the wound and thus a reduction in its concentration at the wound site leads to less EPC recruitment and neovascularization. In summary, diabetes leads to a multitude of events that cause a reduction in angiogenesis, which is essential for wound repair. An agent that remains proangiogenic in hyperglycemia would therefore

be immensely valuable for improving healing in DFUs.

An emerging role for HDL in wound repair

Accumulating evidence demonstrates that HDL display significant wound healing benefits.

For example, dropwise topical application of rHDL to nondiabetic and diabetic wounds in a murine model of wound healing improved the rate of wound closure.^{51,62} Using Laser Doppler imaging, topical HDL was also found to increase wound angiogenesis in both nondiabetic and diabetic mice. This effect of rHDL was attenuated in SR-BI^{-/-} mice, demonstrating that SR-BI, at least in part, mediates improved wound healing and wound angiogenesis by rHDL.⁵¹ Daily topical application of HDL formulated in a 20% Pluronic F-127 gel (PH 7.2) was also found to increase wound healing in atherosclerosis-prone apolipoprotein (apo)E^{-/-} mice through an increase in granulation tissue formation and reepithelialization.⁶³ Furthermore, a study in 24-month-old mice also found that topical rHDL promoted the rate of wound healing and wound angiogenesis.⁶²

Overall, HDL improve wound healing in the context of diabetes, high cholesterol, and aging. The pathways for this are likely to be through an improvement in angiogenesis and an accelerated resolution of inflammation that together assist with wound tissue granulation and reepithelialization.

FUTURE DIRECTIONS

This review provides a comprehensive insight into the currently known evidence on the effects of HDL on inflammation and angiogenesis and highlights its promise as an effective wound healing agent. Future research investigating the efficacy of topical HDL on wound healing in diabetes using *in vitro*, preclinical and clinical testing will facilitate its translation.

SUMMARY

Despite the significant clinical need for effective wound healing therapies with pleiotropic actions, there is still no commonly used therapeutic agent that actively improves healing in chronic wounds such as DFUs. The prevalence of diabetes and the associated complications of diabetes such as DFUs are rising. As discussed in detail, HDL exhibit

TAKE-HOME MESSAGES

- Appropriate inflammation and angiogenesis are essential for optimal wound healing. These processes are impaired in chronic conditions such as diabetes, resulting in nonhealing wounds.
- Currently, there is no effective treatment that actively improves wound healing in diabetes, highlighting a significant unmet clinical need.
- HDL exhibit anti-inflammatory effects in endothelial cells and macrophages, while also promoting proangiogenic functions in endothelial cells. HDL therefore presents as a highly promising therapeutic option for chronic wound healing.
- Preclinical models show that topical HDL can improve wound healing in the contexts of diabetes, atherosclerosis, and aging.

valuable properties such as anti-inflammatory and proangiogenic effects, making it a highly promising therapeutic treatment for wound healing. Future clinical trials that test the effect of topical HDL on acute and chronic wounds are required and eagerly awaited.

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REFERENCES

1. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014;103:137–149.
2. Pena G, Cowled P, Dawson J, Johnson B, Fitridge R. Diabetic foot and lower limb amputations: underestimated problem with a cost to health system and to the patient. *ANZ J Surg* 2018;88:666–667.
3. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med* 2017;376:2367–2375.
4. Okonkwo UA, DiPietro LA. Diabetes and wound angiogenesis. *Int J Mol Sci* 2017;18:1419.
5. Leese GP, Morris AD. Prediction of diabetic foot ulcer occurrence using commonly available clinical information: response to Boyko et al. *Diabetes Care* 2006;29:2562–2563.
6. Blakytyn R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. *Diabet Med* 2006;23:594–608.
7. Game FL, Apelqvist J, Attinger C, et al. Effectiveness of interventions to enhance healing of chronic ulcers of the foot in diabetes: a systematic review. *Diabetes Metabol Res Rev* 2016;32(S1):154–168.
8. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease—the Framingham Heart Study. *Can J Cardiol* 1988;4 Suppl A:5A–10A.
9. Prosser HC, Tan JT, Dunn LL, et al. Multi-functional regulation of angiogenesis by high-density lipoproteins. *Cardiovasc Res* 2014;101:145–154.
10. Murphy AJ, Woollard KJ, Hoang A, et al. High-density lipoprotein reduces the human monocyte inflammatory response. *Arterioscler Thromb Vasc Biol* 2008;28:2071–2077.
11. Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res* 2010;89:219–229.
12. Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing—a literature review. *An Bras Dermatol* 2016;91:614–620.
13. Mirza RE, Fang MM, Ennis WJ, Koh TJ. Blocking interleukin-1 β induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes. *Diabetes* 2013;62:2579–2587.
14. Wong SL, Demers M, Martinod K, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med* 2015;21:815–819.
15. Karima M, Kantarci A, Ohira T, et al. Enhanced superoxide release and elevated protein kinase C activity in neutrophils from diabetic patients: association with periodontitis. *J Leukoc Biol* 2005;78:862–870.
16. Delamaire M, Maugendre D, Moreno M, Le Goff M-C, Allanic H, Genetet B. Impaired leucocyte functions in diabetic patients. *Diabet Med* 1997;14:29–34.
17. Moura J, Rodrigues J, Goncalves M, Amaral C, Lima M, Carvalho E. Impaired T-cell differentiation in diabetic foot ulceration. *Cell Mol Immunol* 2017;14:758–769.
18. Yu CO, Leung KS, Fung KP, et al. The characterization of a full-thickness excision open foot wound model in n5-streptozotocin (STZ)-induced type 2 diabetic rats that mimics diabetic foot ulcer in terms of reduced blood circulation, higher C-reactive protein, elevated inflammation, and reduced cell proliferation. *Exp Anim* 2017;66:259–269.
19. Ferguson MW, Herrick SE, Spencer MJ, Shaw JE, Boulton AJ, Sloan P. The histology of diabetic foot ulcers. *Diabet Med* 1996;13 Suppl 1:S30–S33.
20. Das S, Singh G, Majid M, et al. Syndesome therapeutics for enhancing diabetic wound healing. *Adv Healthc Mater* 2016;5:2248–2260.
21. Muller M, Trocme C, Lardy B, Morel F, Halimi S, Benhamou PY. Matrix metalloproteinases and diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is a predictor of wound healing. *Diabet Med* 2008;25:419–426.
22. Hinchliffe RJ, Forsythe RO, Apelqvist J, et al. Guidelines on diagnosis, prognosis, and management of peripheral artery disease in patients with foot ulcers and diabetes (IWGDF 2019 update). *Diabetes Metabol Res Rev* 2020;36 Suppl 1:e3276.
23. Vas P, Rayman G, Dhatriya K, et al. Effectiveness of interventions to enhance healing of chronic foot ulcers in diabetes: a systematic review. *Diabetes Metabol Res Rev* 2020;36 Suppl 1:e3284.
24. Edmonds M, Lazaro-Martinez JL, Alfayate-Garcia JM, et al. Sucrose octasulfate dressing versus control dressing in patients with neuroischaemic diabetic foot ulcers (Explorer): an international, multicentre, double-blind, randomised, controlled trial. *Lancet Diabetes Endocrinol* 2018;6:186–196.
25. Armstrong DG, Lavery LA, Diabetic Foot Study Consortium. Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomised controlled trial. *Lancet* 2005;366:1704–1710.
26. Uchi H, Igarashi A, Urabe K, et al. Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer. *Eur J Dermatol* 2009;19:461–468.
27. Park KH, Han SH, Hong JP, et al. Topical epidermal growth factor spray for the treatment of chronic diabetic foot ulcers: a phase III multicenter, double-blind, randomized, placebo-controlled trial. *Diabetes Res Clin Pract* 2018;142:335–344.
28. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function recent advances. *J Am Coll Cardiol* 2005;46:1792–1798.
29. Calabresi L, Gomaschi M, Simonelli S, Bernini F, Franceschini G. HDL and atherosclerosis: insights from inherited HDL disorders. *Biochim Biophys Acta* 2015;1851:13–18.
30. Prosser HC, Ng MK, Bursill CA. The role of cholesterol efflux in mechanisms of endothelial protection by HDL. *Curr Opin Lipidol* 2012;23:182–189.
31. Murphy AJ, Woollard KJ. High-density lipoprotein: a potent inhibitor of inflammation. *Clin Exp Pharmacol Physiol* 2010;37:710–718.
32. Arca M, Montali A, Valiante S, et al. Usefulness of atherogenic dyslipidemia for predicting cardiovascular risk in patients with angiographically defined coronary artery disease. *Am J Cardiol* 2007;100:1511–1516.
33. Femlak M, Gluba-Brzozka A, Cialkowska-Rysz A, Rysz J. The role and function of HDL in patients with diabetes mellitus and the related cardiovascular risk. *Lipids Health Dis* 2017;16:207.
34. Chen Y, Zhi Y, Li C, et al. HDL cholesterol and risk of diabetic nephropathy in patient with type 1 diabetes: a meta-analysis of cohort studies. *Diabetes Res Clin Pract* 2016;122:84–91.
35. Ikura K, Hanai K, Shinjyo T, Uchigata Y. HDL cholesterol as a predictor for the incidence of lower extremity amputation and wound-related death in patients with diabetic foot ulcers. *Atherosclerosis* 2015;239:465–469.
36. Kimura T, Tomura H, Mogi C, et al. Role of scavenger receptor class B type I and sphingosine 1-phosphate receptors in high density lipoprotein-induced inhibition of adhesion molecule expression in endothelial cells. *J Biol Chem* 2006;281:37457–37467.
37. Bursill CA, Castro ML, Beattie DT, et al. High-density lipoproteins suppress chemokines and chemokine receptors in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 2010;30:1773–1778.
38. Nicholls SJ, Dusting GJ, Cutri B, et al. Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. *Circulation* 2005;111:1543–1550.
39. Van Lenten BJ, Navab M, Shih D, Fogelman AM, Lusis AJ. The role of high-density lipoproteins in oxidation and inflammation. *Trends Cardiovasc Med* 2001;11:155–161.
40. Boniakowski AE, Kimball AS, Jacobs BN, Kunkel SL, Gallagher KA. Macrophage-mediated inflammation in normal and diabetic wound healing. *J Immunol* 2017;199:17–24.

41. Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. *Nat Rev Immunol* 2010;10:453–460.
42. Dal-Secco D, Wang J, Zeng Z, et al. A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2+ monocytes at a site of sterile injury. *J Exp Med* 2015;212:447–456.
43. Sanson M, Distel E, Fisher EA. HDL induces the expression of the M2 macrophage markers arginase 1 and Fizz-1 in a STAT6-dependent process. *PLoS One* 2013;8:e74676.
44. Liu T, Dhanasekaran SM, Jin H, et al. FIZZ1 stimulation of myofibroblast differentiation. *Am J Pathol* 2004;164:1315–1326.
45. Burger D, Dayer JM. High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev* 2002;1:111–117.
46. De Nardo D, Labzin LI, Kono H, et al. High-density lipoprotein mediates anti-inflammatory reprogramming of macrophages via the transcriptional regulator ATF3. *Nat Immunol* 2014;15:152–160.
47. MacLeod AS, Mansbridge JN. The innate immune system in acute and chronic wounds. *Adv Wound Care (New Rochelle)* 2016;5:65–78.
48. Arnold F, West DC. Angiogenesis in wound healing. *Pharmacol Ther* 1991;52:407–422.
49. Tan JTM, Ng MKC, Bursill CA. The role of high-density lipoproteins in the regulation of angiogenesis. *Cardiovasc Res* 2015;106:184–193.
50. Seetharam D, Mineo C, Gormley AK, et al. High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B type I. *Circ Res* 2006;98:63–72.
51. Tan JT, Prosser HC, Dunn LL, et al. High-density lipoproteins rescue diabetes-impaired angiogenesis via scavenger receptor class B type I. *Diabetes* 2016;65:3091–3103.
52. Tan JT, Prosser HC, Vanags LZ, Monger SA, Ng MK, Bursill CA. High-density lipoproteins augment hypoxia-induced angiogenesis via regulation of post-translational modulation of hypoxia-inducible factor 1 α . *FASEB J* 2014;28:206–217.
53. Primer KR, Psaltis PJ, Tan JTM, Bursill CA. The role of high-density lipoproteins in endothelial cell metabolism and diabetes-impaired angiogenesis. *Int J Mol Sci* 2020;21:10.
54. Cannizzo CM, Adonopulos AA, Solly EL, et al. VEGFR2 is activated by high-density lipoproteins and plays a key role in the proangiogenic action of HDL in ischemia. *FASEB J* 2018;32:2911–2922.
55. Jin F, Hagemann N, Sun L, et al. High-density lipoprotein (HDL) promotes angiogenesis via S1P3-dependent VEGFR2 activation. *Angiogenesis* 2018;21:381–394.
56. Miura S, Fujino M, Matsuo Y, et al. High density lipoprotein-induced angiogenesis requires the activation of Ras/MAP kinase in human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol* 2003;23:802–808.
57. Waltenberger J, Lange J, Kranz A. Vascular endothelial growth factor-A-induced chemotaxis of monocytes is attenuated in patients with diabetes mellitus: a potential predictor for the individual capacity to develop collaterals. *Circulation* 2000;102:185–190.
58. Seitz O, Schurmann C, Hermes N, et al. Wound healing in mice with high-fat diet- or ob gene-induced diabetes-obesity syndromes: a comparative study. *Exp Diabetes Res* 2010;2010:476969.
59. Qi W, Yang C, Dai Z, et al. High levels of pigment epithelium-derived factor in diabetes impair wound healing through suppression of Wnt signaling. *Diabetes* 2015;64:1407–1419.
60. Eming SA, Koch M, Krieger A, et al. Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. *J Proteome Res* 2010;9:4758–4766.
61. Eming SA, Lauer G, Cole M, et al. Increased levels of the soluble variant of the vascular endothelial growth factor receptor VEGFR-1 are associated with a poor prognosis in wound healing. *J Invest Dermatol* 2004;123:799–802.
62. Tsatralis T, Ridiandries A, Robertson S, et al. Reconstituted high-density lipoproteins promote wound repair and blood flow recovery in response to ischemia in aged mice. *Lipids Health Dis* 2016;15:150.
63. Gordts SC, Muthuramu I, Amin R, Jacobs F, De Geest B. The impact of lipoproteins on wound healing: topical HDL therapy corrects delayed wound healing in apolipoprotein E deficient mice. *Pharmaceuticals (Basel)* 2014;7:419–432.
64. Yuhanna IS, Zhu Y, Cox BE, et al. High-density lipoprotein binding to scavenger receptor-B1 activates endothelial nitric oxide synthase. *Nat Med* 2001;7:853–857.

Abbreviations and Acronyms

CETP	=	cholesterol ester transfer protein
DFU	=	diabetic foot ulcer
EGF	=	epithelial growth factor
eNOS	=	endothelial nitric oxide synthase
FGF	=	fibroblast growth factor
HDL	=	high-density lipoprotein
ICAM	=	intercellular cell adhesion molecule
IGF	=	insulin-like growth factor
IL-1 β	=	interleukin beta 1
MMP	=	matrix metalloproteinase
NF- κ B	=	nuclear factor kappa B
NGF	=	nerve growth factor
NO	=	nitric oxide
PDGF	=	platelet-derived growth factor
PHD	=	prolyl hydroxylase domain
PMA	=	phorbol 12-myristate-13-acetate
ROS	=	reactive oxygen species
S1P3	=	sphingosine-1 phosphate receptor 3
SDF	=	stromal cell-derived factor
TGF- β	=	transforming growth factor- β
TLR	=	Toll-like receptor
TNF- α	=	tumor necrosis factor- α
VCAM-1	=	vascular cell adhesion molecule-1
VEGF	=	vascular endothelial growth factor