Systematic review and meta-analysis of mouse models of diabetes-associated ulcers

Pacific Huynh, James Phie, Smriti Murali Krishna, Jonathan Golledge

ABSTRACT

Mouse models are frequently used to study diabetes-associated ulcers, however, whether these models accurately simulate impaired wound healing has not been thoroughly investigated. This systematic review aimed to determine whether wound healing is impaired in mouse models of diabetes and assess the quality of the past research. A systematic literature search was performed of publicly available databases to identify original articles examining wound healing in mouse models of diabetes. A meta-analysis was performed to examine the effect of diabetes on wound healing rate using random effect models. A meta-regression was performed to examine the effect of diabetes duration on wound healing impairment. The quality of the included studies was also assessed using two newly developed tools. 77 studies using eight different models of diabetes within 678 non-diabetic and 720 diabetic mice were included. Meta-analysis showed that wound healing was impaired in all eight models. Meta-regression suggested that longer duration of diabetes prior to wound induction was correlated with greater degree of wound healing impairment. Pairwise comparisons suggested that non-obese diabetic mice exhibited more severe wound healing impairment compared with db/db mice, streptozotocin-induced diabetic mice or high-fat fed mice at an intermediate stage of wound healing (p<0.01). Quality assessment suggested that the prior research frequently lacked incorporation of key clinically relevant characteristics. This systematic review suggested that impaired wound healing can be simulated in many different mouse models of diabetes but these require further refinement to become more clinically relevant.

INTRODUCTION

Diabetes-related foot disease (DFD) is a leading cause of impaired health-related quality of life, amputation, hospitalization and healthcare costs.1–6 The most common presentation of DFD is a foot ulcer which is estimated to develop in 10–20 million people worldwide annually.1 The lifetime incidence of foot ulceration in people with diabetes has been estimated as up to 30%.1 4 The mortality of patients with diabetes-related foot ulcers has been estimated to be 50% over 5 years and more than double that of people with diabetes but no foot ulcer.1 7 8 The development of improved understanding of diabetes-related ulcer pathogenesis and the discovery of novel treatments are therefore global priorities.

Animal models of human disease are commonly used to identify new treatments. There are many methods of inducing diabetes that have been used to study atherosclerosis, nephropathy and neuropathy in mice.9–11 There has, however, been limited focus on modeling DFD in mice. There is no current consensus on the most appropriate mouse model of diabetes-associated ulceration. A key requirement of a model of diabetes-associated ulceration is impaired wound healing. This has been attributed to several pathological processes stimulated by chronic hyperglycemia, including atherosclerosis and microvascular disease that lead to leg ischemia and peripheral neuropathy.12 13 It is not currently clear which of the different methods of inducing diabetes in mice are associated with wound healing impairment.

In order to inform the appropriate choice of animal model, this review aimed to systematically examine the healing rates of wounds in mouse models of diabetes. The review also examined the quality and clinical relevance of this past research.

METHODS

Search strategy, inclusion and exclusion criteria

This review was performed according to the 2015 Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols statement (online supplementary table 1).14 The protocol was registered in the PROSPERO database (Registration Number: CRD42018116224). Searches of the literature were conducted between 16 August 2018 and 24 January 2019 by one author (PH) on three separate occasions. The databases Medline, PubMed, Scopus, ScienceDirect and Web of Science were searched to identify preclinical studies examining the effects of diabetes on wound healing in mice. The
full search strategy consisted of the terms (‘diabetic wound’ OR ‘diabetic foot ulcer’) AND (mouse OR mice OR murine) AND (nondiabetic OR non-diabetic OR normoglycemi* OR normoglycaemi*) using both Medical Subject Headings and keyword searches. The resultant articles were filtered in two stages, automatically using the database filters and manually by one author (PH) to only include original journal articles written in English. For inclusion, studies had to examine wound healing in mouse models of diabetes compared with age-matched controls and report wound area as a ratio or percentage of the initial wound area monitored over time for both groups. 

**Data extraction and quality assessment of included studies**

Full-text publications of included studies were independently assessed by two investigators (PH and JP). Data extracted included sex, age and strain of mice, model of diabetes, diagnostic criteria for diabetes, whether diabetes was confirmed prior to wound generation, the size and location of the initial wound, method and period of monitoring, and wound closure percentage defined as detailed below. ImageJ V.1.48 (National Institutes of Health, USA) was used to extrapolate data from figures if required. Extracted data were discussed in a consensus meeting.

The quality of the included studies was assessed using two tools specifically developed for this systematic review (online supplementary tables 2 and 3). One tool assessed the study design and reporting quality of the studies and was based on a prior tool,15 and the Animal Research: Reporting of In Vivo Experiments guidelines (online supplementary table 2). The second tool assessed the design of the mouse model experiment in relation to recognized characteristic features of human diabetes-associated ulcers (online supplementary table 3). Each checklist item was weighted equally and graded 0, 0.5 or 1 for no, unclear or yes, respectively. Each study was scored as a percentage of the total possible score for each tool.

**Data analysis**

The primary outcome of this systematic review and meta-analysis was percentage (%) wound closure which was calculated from extracted data using the following formula:

\[
\text{% Wound Closure} = \left(1 - \frac{\text{Wound Area}}{\text{Initial Wound Area}}\right) \times 100%
\]

To gauge the effect of diabetes on wound closure over time, extracted data were sorted for analysis to early (2–5 days), intermediate (6–10 days) and late stages (11–20 days) of wound healing. In the event that a single article contained multiple independent animal studies, wound closure data were extracted from each of these studies and treated as independent data. If an article appeared to have multiple animal studies using the same mice in their experimental groups, sample sizes were evenly divided among them for meta-analysis to minimize duplication of data. For example, if primary outcome data were available for two different sets of diabetic mice but the authors used the same mice as controls for both, the sample size of the control group was evenly divided between the two diabetic groups for analysis. Corresponding authors of studies were contacted for key missing data. When necessary, SEMs were converted to SDs using GraphPad Prism V.7 (San Diego, California, USA). Meta-analyses were performed to assess the effect of diabetes on wound closure using Review Manager V.5.3.5 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), while meta-regression analyses were performed using Open Meta-Analyst.16 Since heterogeneity between studies was expected, random effects models were used. Data were reported as standardized mean difference (SMD) with 95% CIs. Subgroup analyses were performed to examine whether the extent of wound healing impairment was different between the models of diabetes included. These pairwise comparisons were corrected for multiple testing using Bonferroni’s correction. Leave-one-out sensitivity analyses were also performed. The I² index was used to assess the degree of heterogeneity between studies, with I² >50% accepted to denote statistical heterogeneity. Funnel plots of the effect size versus the SEM of the log-transformed effect were constructed to assess potential publication bias. A p value <0.05 was considered to be statistically significant.

**RESULTS**

**Study selection**

After a systematic search, a total of 77 studies were included in this systematic review and meta-analysis (online supplementary figure 1).17-93

**Characteristics of the included studies**

The characteristics of the 77 included studies are shown in online supplementary table 4. There were a total of eight different mouse models of diabetes used (with three studies using multiple models).62 68 89 These included streptozotocin (STZ) injection (n=41; 20 single dose; 21 multiple doses), leptin receptor defective db/db mice (n=27), alloxan injection (n=4; 3 single dose; 1 multiple doses), high-fat fed mice (n=4), leptin-deficient ob/ob mice (n=2) and non-obese diabetic (NOD) mice (n=2). The majority of studies reported including male mice only (49/77). Eleven investigations included female mice only, two included mice of both sexes and the remaining studies did not report the sex of the mice included (15/77).

Only three studies examined wounds created in the hindlimb.37 51 61 The other studies investigated wounds created on the torso of mice (74 in total; 71 on the back, 3 on the flank). The initial wound size differed greatly between studies (online supplementary table 4). Ten studies used splints to prevent the closure of wounds via contraction and promote wound healing through re-epithelialization since this has been suggested to be more typical of humans.94 95
Quality of design and reporting of the included studies

The mean study design quality assessment score was 55.1% (range 19.2%–80.8%) (online supplementary table 5). Most studies (n=73) provided an ethics statement. Only nine studies reported that examination of wound closure was performed by an assessor blinded to group allocation. Only two studies indicated that investigators were blinded to the experimental groups of mice.17,72 Only three studies reported that power calculations were performed to determine appropriate sample sizes,22 37 47 while an additional two justified their sample sizes based on previous experiments.63 72 Forty-one studies did not report full information about the strain of mice used. Twelve studies did not indicate the start date of their studies,20 40–44 48 54 55 57 80 84 with an additional 27 only reporting the age of mice as ranges.17–19 21 23 24 26 28 29 36 39 45–53 60 62 73 78 81 87–90 93 Eighteen studies which investigated STZ-induced diabetes did not indicate whether non-diabetic mice were injected with vehicle.27 29 30 33 35 37 38 40 47 49 51 52 53 54 60–62 68 71 73 74 76 78 83 In studies which used genetic models of diabetes, 14 used homozygote mice of the background strain or non-diabetic littermates, as controls mice.19–21 23 24 29 45 46 50 52 70 81 82 89 while two studies did not indicate the use of littermate controls.40 56 Two studies described the wound closure measurements in the methods but represented their data in another way.70 85

Relevance of the reported mouse models to human diabetes-associated ulceration

The mean clinical relevance quality assessment score was 44.9% (range 26.9%–65.4%) (online supplementary table 6). Most studies (n=46) reported a clear diagnostic criterion for diabetes. This was mainly based on blood glucose concentrations. One study reported the confirmation of diabetes by the presence of glycosuria.20 Despite the Diabetic Complications Consortium recommendations for the appropriate confirmation of diabetes in animal models, only 15 of the 46 studies reported blood glucose measurements were performed in fasted mice.18 25 33 35 38 65–69 72 76 77 81 91 and only three reported monitoring additional diabetes-associated metabolic parameters, with glycosuria being the common parameter measured.25 58 61 Furthermore, only 16 studies extensively reported blood glucose levels of included mice.33 35 38 42 49 56 60 63–65 69 74 77 81 87 91 Only nine studies reported the time required for complete wound healing.18 50 52 62 63 80 84 88 90 Four studies reported measures of wound blood supply,49 69 71 87 with an additional study only reporting perfusion in a subset of mice.29 No investigation reported signs of neuropathy.

The effect of diabetes on wound closure

Meta-analyses included data extracted to assess the effect of diabetes on wound closure at early (n=1346), intermediate (n=1398) and late (n=870) stages of wound healing. Diabetes led to impairment of wound closure at all stages (SMD 1.25 (95% CI 1.01 to 1.48, p<0.001), 2.28 (95% CI 1.94 to 2.62, p<0.001) and 3.12 (95% CI 2.66 to 3.59, p<0.001) at early, intermediate and late stages, respectively (figures 1–3 and online supplementary tables 7–9)). There was substantial statistical heterogeneity between studies (I²=70%–80%, figures 1–3 and online supplementary tables 7–9).

Subgroup analyses suggested that wound closure was significantly impaired when compared with non-diabetic mice at all stages of wound healing in all models of diabetes (figures 1–3 and online supplementary tables 7–9) except in the high-fat fed mice and ob/ob mice at the early stage of wound healing (SMD −0.17 (95% CI −0.89 to 1.23), and SMD 3.55 (95% CI −0.07 to 7.18), respectively) (figure 1 and online supplementary table 7). Pairwise comparisons suggested significant differences in the degree of wound healing impairment in the different diabetes models (online supplementary table 10). db/db mice had greater wound healing impairment than found in the multiple-dose STZ-induced and high-fat fed models at intermediate and late stages of wound healing (online supplementary table 10). The single-dose STZ-induced diabetes model had more severe wound healing impairment than the multiple-dose STZ-induced diabetes model at late stages of wound healing (online supplementary table 10).

Meta-regression suggested that a longer duration of diabetes prior to wound generation was associated with greater impairment of wound healing (online supplementary figure 2c, p=0.021 at late stages after removal of statistical outliers). Leave-one-out sensitivity analyses suggested that all studies contributed towards the main findings in the meta-analysis (online supplementary figure 3). Funnel plots suggested potential publication bias (online supplementary figure 4).

DISCUSSION

This systematic review suggests that mouse models of diabetes consistently have impaired wound healing. The wound healing impairment was clearer as the time after wound induction increased. The severity of wound healing impairment varied between the different mouse models. The meta-regression suggested a tendency towards greater wound healing impairment with longer duration of hyperglycemia. There was substantial statistical heterogeneity and limited reporting of important information in the included studies. Furthermore, very few studies modeled some of the common characteristics of human diabetes-associated ulcers, such as location in the periphery of the limb and concurrent ischemia and neuropathy (online supplementary table 11). These findings highlight the need for more clinically relevant models of diabetes-associated ulceration.

Eight different methods of modeling diabetes were used in the studies included in this systematic review (summarized in table 1, sorted from most to least severe wound healing impairment). Meta-analysis of data reported early after wound induction suggested that the
Figure 1  Forest plots showing the effect of diabetes on the early stages of wound closure in different mouse models of diabetes. Forest plots were generated from Review Manager V.5.3 to represent early (2–5 days) stages of wound closure. Comparisons were made using standard mean differences and random effects models.
Figure 2 Forest plots showing the effect of diabetes on the intermediate stages of wound closure in different mouse models of diabetes. Forest plots were generated from Review Manager V.5.3 to represent intermediate (6–10 days) stages of wound closure. Comparisons were made using standard mean differences and random effects models.
Figure 3 Forest plots showing the effect of diabetes on the late stages of wound closure in different mouse models of diabetes. Forest plots were generated from Review Manager V.5.3 to represent late (11–20 days) stages of wound closure. Comparisons were made using standard mean differences and random effects models.
variable onset of diabetes. Nonetheless, these models to
toxicity and lower effectiveness,
able as a diabetogenic agent to STZ due to greater organ
For example, alloxan is generally considered less favor-
the more commonly used mouse models of diabetes.
Second, these models have disadvantages compared with
models simulate type 1 rather than type 2 diabetes.
models is likely due to a number of factors. First, both
diabetes and NOD models. The infrequent use of these
suggested that NOD mice had the
most severe impairment of wound healing. These find-
ings, however, should be interpreted cautiously given the
small and uneven number of studies which reported the
use of these models. Indeed, only a total of three studies
reported the use of the multiple-dose alloxan-induced
diabetes and NOD models. The infrequent use of these
models is likely due to a number of factors. First, both
models simulate type 1 rather than type 2 diabetes,
which is the less common form of diabetes in people. Second,
these models have disadvantages compared with
the more commonly used mouse models of diabetes.
For example, alloxan is generally considered less favor-
able as a diabetogenic agent to STZ due to greater organ
toxicity and lower effectiveness, while NOD mice have
variable onset of diabetes. Nonetheless, these models
do appear to simulate diabetes-associated wound healing
impairment. High-fat feeding is an approach used to
simulate type 2 diabetes. This review suggested that this
model had the least severe wound healing impairment of
all the models examined, possibly due to its milder
metabolic derangement. Meta-analyses of data obtained
late after wound induction also suggested that impair-
ment of wound healing was more severe in the single-
dose STZ-induced diabetes model compared with the
multiple-dose STZ-induced diabetes model. It should be
noted though that there were substantial methodological
differences between the included studies. These compar-
isons between models should therefore be interpreted
very cautiously.

It was surprising to find limited reporting of relevant
metabolic parameters and data to confirm the diagnos-
is of diabetes within the included studies. Suscepti-
bility towards diabetes-associated complications in mice
is dependent on a number of different factors, such as
age, strain and sex. Furthermore, studies focusing
on other diabetic complications, such as atherosclerosis
and neuropathy, typically require an extended duration
of diabetes, as well as genetic manipulation, to simulate
these complications. In the meta-regression,
there was a trend towards greater wound healing impair-
ment with longer duration of hyperglycemia before
wound induction (online supplementary figure 2). Many
of the studies with a relatively long duration of diabetes
(>6 weeks) used genetic models which may have influ-
enced the findings of the meta-regression. Nonetheless,
investigators should take into consideration the severity
and duration of diabetes required to simulate the clinical
presentation.

While limbs are the main site of diabetes-associated
ulcers in patients, all but three studies included in this
systematic review examined wounds generated on the
torso of mice. The greater area on the torso allows
multiple wounds to be studied but it is unlikely such ulcers
simulate the clinical situation. Peripheral artery disease

### Table 1: Mouse models of diabetes-associated ulceration ordered in relation to the severity of wound healing impairment

| No | Early stage (2–5 days) | Intermediate stage (6–10 days) | Late stage (11–20 days) |
|----|------------------------|--------------------------------|------------------------|
| 1  | Multiple-dose alloxan  | NOD SMD 6.09 (95% CI: 4.47 to 7.72) | NOD SMD 14.59 (95% CI: 4.46 to 24.71) |
|    | SMD 5.35 (95% CI: 2.50 to 8.20) | n=6 Con, 6 Dia | n=4 Con, 4 Dia |
|    | n=6 Con, 6 Dia | | |
| 2  | ob/ob  | SMD 3.55 (95% CI: −0.07 to 7.18) | Multiple-dose alloxan |
|    | SMD 3.55 (95% CI: −0.07 to 7.18) | n=8 Con, 8 Dia | SMD 5.84 (95% CI: 2.77 to 8.91) |
|    | n=8 Con, 8 Dia | | n=6 Con, 6 Dia |
| 3  | Single-dose STZ  | SMD 5.89 (95% CI: 2.80 to 8.98) | Single-dose STZ |
|    | SMD 1.53 (95% CI: 0.96 to 2.10) | n=205 Con, 205 Dia | SMD 3.58 (95% CI: 2.57 to 4.59) |
|    | n=205 Con, 205 Dia | | n=114 Con, 114 Dia |
| 4  | NOD  | SMD 4.57 (95% CI: 2.1 to 7.04) | Single-dose STZ |
|    | SMD 1.47 (95% CI: 0.68 to 2.27) | n=8 Con, 8 Dia | SMD 3.87 (95% CI: 3.11 to 4.63) |
|    | n=18 Con, 14 Dia | | n=186 Con, 215 Dia |
| 5  | Single-dose alloxan  | SMD 2.9 (95% CI: 2.29 to 3.52) | Single-dose STZ |
|    | SMD 1.20 (95% CI: 0.42 to 1.99) | n=212 Con, 240 Dia | SMD 2.05 (95% CI: 0.38 to 3.72) |
|    | n=17 Con, 17 Dia | | n=17 Con, 17 Dia |
| 6  | db/db  | SMD 2.32 (95% CI: 0.63 to 4.01) | Single-dose STZ |
|    | SMD 1.18 (95% CI: 0.76 to 1.6) | n=196 Con, 198 Dia | SMD 2.16 (95% CI: 1.06 to 1.99) |
|    | n=206 Con, 232 Dia | | n=69 Con, 76 Dia |
| 7  | Multiple-dose STZ  | SMD 1.69 (95% CI: 1.27 to 2.11) | Multiple-dose STZ |
|    | SMD 1.16 (95% CI: 0.84 to 1.47) | n=183 Con, 195 Dia | SMD 1.53 (95% CI: 0.81 to 2.26) |
|    | n=178 Con, 184 Dia | | n=69 Con, 76 Dia |
| 8  | High-fat fed  | SMD 1.16 (95% CI: 0.38 to 1.95) | High-fat fed |
|    | SMD 0.17 (95% CI: −0.89 to 1.23) | n=34 Con, 38 Dia | SMD 1.34 (95% CI: 0.65 to 2.03) |
|    | n=19 Con, 23 Dia | | n=19 Con, 23 Dia |

* P<0.01, significant differences as determined by pairwise comparison using Bonferroni’s correction (online supplementary table 10).
Con, non-diabetic control; Dia, diabetic; NOD, non-obese diabetic; SMD, standardized mean difference; STZ, streptozotocin.
and neuropathy are key causes of ulcers in people. Only four of the included studies examined blood flow within the mice. None of the included studies artificially induced artery disease or neuropathy within the mice. Artificial generation of peripheral ischemia or neuropathy is likely required to simulate the clinical presentation since mice are very resistant to development of diabetes-associated microvascular complications. Use of genetically modified mice may also be used to simulate other clinically relevant risk factors, such as dyslipidemia which accelerates diabetes-associated atherosclerosis and neuropathy. Table 2 presents suggested criteria for future diabetes-associated ulcer studies in rodents aimed to improve the clinical relevance of the research.

### Limitations and strengths

To the best of our knowledge, this is the first systematic review and meta-analysis examining wound healing impairment in different mouse models of diabetes. The current study used a range of analysis methods and quality assessment methods in order to rigorously assess past research. The included studies had many quality weaknesses such as small sample sizes. There was also absence of key study design and reporting features, like blinding of outcome assessors and inclusion of sample size calculations. Many of the included studies did not report parameters relevant to diabetes or determined the severity of diabetes. Therefore, it was not possible to systematically assess the correlation between glucose control and wound healing impairment. There was also statistical and methodological heterogeneity between studies. Due to the heterogeneous and intermittent reporting of outcomes, it was not possible to systematically examine the molecular mechanisms involved in wound healing impairment. Nonetheless, the findings from this study provide important insight into the strengths and weaknesses of current mice models of diabetes-associated ulcers.

### CONCLUSION

In conclusion, this systematic review suggests that, regardless of diabetes induction method, hyperglycemia impairs wound healing in mice. Incorporation of clinically relevant aspects, such as limb ischemia and prolonged diabetes duration, may improve the translation of findings from the mouse models.

### Acknowledgements

We thank Emeritus Professor Rhondda Jones for her statistical advice.

### Contributors

PH, JP, SMK and JG were responsible for the conceptualization of the review, with data curation and analysis performed by PH and JP. The writing of this manuscript was performed by PH and JG.

### Funding

This work was supported by James Cook University’s Strategic Research Intent Fund. JG holds a National Health and Medical Research Council (NHMRC) Practitioner Fellowship (1117061) and Queensland Government Senior Clinical Research Fellowship (SCRF).

### Competing interests

None declared.

### Patient consent for publication

Not required.

### Provenance and peer review

Not commissioned; externally peer reviewed.

### Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information.

### Open access

This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

### ORCID iDs

Pacific Huynh http://orcid.org/0000-0001-6729-2296
Jonathan Golledge http://orcid.org/0000-0002-5779-8848

### REFERENCES

1. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med* 2017;376:2367–75.
2. Boulton AJM, Vileikyte L, Ragnarson-Tennvall G, et al. The global burden of diabetic foot disease. *Lancet* 2005;366:1719–24.
3. Lazzarini PA, Hurn SE, Fernando ME, et al. Prevalence of foot disease and risk factors in general inpatient populations: a systematic review and meta-analysis. *BMJ Open* 2015;5:e008544.
4. Boulton AJM, Kirsnser RS, Vileikyte L. Neuropathic diabetic foot ulcers. *N Engl J Med Overseas Ed* 2004;351:48–55.
5. Baker IDI Heart and Diabetes Institute. National evidence-based guideline on prevention, identification and management of foot complications in diabetes. Melbourne Australia, 2011.
6. Lazzarini PA, Pacella RE, Armstrong DG, et al. Diabetes-Related lower-extremity complications are a leading cause of the global burden of disability. *Diabet Med* 2018. doi:10.1111/dme.13880. [Epub ahead of print: 23 May 2018].
Abu-Al-Basal MA. Healing potential of Rosmarinus officinalis L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. J Ethnopharmacol 2010;131:443–50.

Fang Y, Shen J, Yao M, et al. Granulocyte-Macrophage colony-stimulating factor enhances wound healing in diabetes via upregulation of proinflammatory cytokines. Br J Dermatol 2010;162:478–86.

Jacobsen JN, Steffensen B, Häkkinen L, et al. Skin wound healing in diabetic [6 integrin-deficient mice. AMPIS 2010;118:753–64.

Marotte EJ, Chen D-D, Hakim JS, et al. Manganese superoxide dismutase expression in endothelial progenitor cells accelerates wound healing in diabetic mice. J Clin Invest 2010;120:4207–19.

Aalbro M, Menegazzio L, Boscaro E, et al. Defective recruitment, survival and proliferation of bone marrow-derived progenitor cells at sites of delayed diabetic wound healing in mice. Diabetologia 2011;54:945–53.

Hegde VN, Prabhu V, Rao SBS, et al. Effect of laser dose and treatment schedule on excision wound healing in diabetic mice. Photochem Photobiol 2011;87:1433–41.

Mirza R, Koh TJ. Dysregulation of monocyte/macrophage phenotype in wounds of diabetic mice. Cytokeine 2011;56:256–64.

Wetterau M, George F, Weinstein A, et al. Topical prolyl hydroxylase domain-2 silencing improves diabetic murine wound closure. Wound Repair Regen 2011;19:481–6.

Badr G. Supplementation with undenatured whey protein during diabetes mellitus improves the healing and closure of diabetic wounds through the rescue of functional long-lived wound macrophages. Cell Physiol Biochem 2012;29:571–82.

Badr G, Badr BM, Mahmoud NH, et al. Treatment of diabetic mice with undenatured whey protein accelerates the wound healing process by enhancing the expression of MIP-1α, MIP-2, KC, CXCCL1 and TGF-β in wounded tissue. BMC Immunol 2012;13:32.

Loyd CM, Diaconu D, Fu W, et al. Transgenic overexpression of keratinocyte-specific VEGF and Ang1 in combination promotes wound healing under nondiabetic but not diabetic conditions. Int J Clin Exp Pathol 2012;5:1–11.

Mohany M, Badr BM, Mahmoud M, et al. Un-denatured whey protein expedites wound healing in diabetic mice model by enhancing the expression of interleukin 3, 2, 3 and vascular endothelial growth factor (VEGF) in the wounded tissue. Afl J Microbiol Res 2012;6:2137–44.

Shin L, Peterson DA. Impaired therapeutic capacity of autologous stem cells in a model of type 2 diabetes. Stem Cells Transl Med 2012;1:125–35.

Steinauresser L, Hirsch T, Schulte M, et al. Innate defense regulator peptide 1018 in wound healing and wound infection. PLoS One 2012;7:e93973.

Wagner IJ, Szpalski C, Allen RJ, et al. Obesity impairs wound closure through a vasculogenic mechanism. Wound Repair Regen 2012;20:1a–22.

Badr G. Camel whey protein enhances diabetic wound healing in a streptozotocin-induced diabetic mouse model: the critical role of HIF-1α/Fn-1, -2 and -3. Lipids Health Dis 2013;12:46.

Tie L, An Y, Han J, et al. HIF-1α silencing accelerates refractory wound healing by suppressing superoxide and FoxO1/NOS pathway in type 1 diabetes. Nutr Biochem 2013;24:88–96.

Dhill S, Do DC, Garcia M, et al. Generating and reversing chronic wounds in diabetic mice by manipulating wound redox parameters. J Diabetes Res 2014;2014:562652.

Fadini GP, Albiero M, Millioni R, et al. The molecular signature of impaired diabetic wound healing identifies serpinB3 as a healing biomarker. Diabetologia 2014;57:1947–56.

Gooyst M, Peng Z, Wolter WR, et al. An angiobiological strategy to facilitate diabetic wound healing. ACS Chem Biol 2014;9:105–10.

Fiu C, Chen D-D, Sun X, et al. Hydrogen sulfide improves wound healing via restoration of endothelial progenitor cell functions and activation of angiopoietin-1 in type 2 diabetes. Diabetes 2014;63:1763–70.

Moura LIF, Dias AMA, Leal EC, et al. Chitosan-based dressings loaded with neurotransin—an efficient strategy to improve early diabetic wound healing. Acta Biomater 2014;10:843–57.

Moura LIF, Dias AMA, Suesca E, et al. Neurotensin-loaded collagen dressings reduce inflammation and improve wound healing in diabetic mice. Biochip Biophys Acta 2014;1842:32–43.

Steinauer L, Lam MC, Jacobsen F, et al. Skin electroporation of a plasmid encoding hCAP-18/LL-37 host defense peptide promotes wound healing. Mol Ther 2014;22:734–42.

Wang Q-Q, Lee S, Sharner H, et al. Sustained transfection of GM3 depletion reverses impaired wound healing in diabetic mice by activating IGF-1 and insulin receptors. J Invest Dermatol 2014;134:1446–55.
et al. Interleukin-22 promotes wound repair in diabetes by improving keratinocyte pro-healing functions. *J Invest Dermatol* 2015;135:2862–70.

Hozzein WN, Badr G, Al Ghamdi AA, et al. Topical application of propolis enhances cutaneous wound healing by promoting TGFBeta/Smad-mediated collagen production in a streptozotocin-induced type I diabetic mouse model. *Cell Physiol Biochem* 2015;37:940–54.

Leal EC, Carvalho E, Tellechea A, et al. Substance P promotes wound healing in diabetic mice by modulating inflammation and macrophage phenotype. *Am J Pathol* 2015;185:1638–48.

Lim Y-G, Bhatt MP, Kwon M-H, et al. Proinulin C-peptide prevents impaired wound healing by activating angiogenesis in diabetes. *J Invest Dermatol* 2015;135:289–78.

Rebalka IA, Raleigh MJ, D’Souza DM, et al. Inhibition of PAI-1 via PAI-039 improves dermal wound closure in diabetes. *Diabetes* 2015;64:2593–602.

Wong SL, Demers M, Martinod K, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med* 2015;21:815–6.

Badr G, Hozzein WN, Badr BM, et al. Bee Venom Accelerates Wound Healing in Diabetic Mice by Suppressing Activating Transcription Factor-3 (ATF-3) and Inducible Nitric Oxide Synthase (iNOS)-mediated Oxidative Stress and Recruiting Bone Marrow-Derived Endothelial Progenitor Cells. *J Cell Physiol* 2016;231:2159–71.

Desposito D, Chollet C, Taveau C, et al. Improvement of skin wound healing in diabetic mice by kinin B2 receptor blockade. *Clin Transl Sci* 2016;13:165–72.

Dong M-W, Li M, Chen J, et al. Activation of autophagy promotes diabetic wound healing by suppressing age-induced TNF-α production. *Inflammation* 2016;39:687–99.

Ho E, Lee H-J, Lim Y. Ameliorative effect of dietary genistein on diabetes induced hyper-inflammation and oxidative stress during early stage of wound healing in alloxan induced diabetic mice. *Biochim Biophys Acta* 2016;1868:2481–94.

Wang F, Liu B, Yu Z, et al. Effects of CD100 promote wound healing in diabetic mice. *J Mol Histol* 2018;49:277–87.

Yan J, Tie G, Wang S, et al. Diabetes impairs wound healing by DNmt1-dependent demethylation of histones H3K9me2 in macrophages differentiation towards macrophages. *Nat Commun* 2018;9:33.

Yu B, Alboslermy T, Safadi F, et al. Glycoprotein nonmelanoma clone B regulates the crosstalk between macrophages and mesenchymal stem cells toward wound repair. *J Invest Dermatol* 2018;138:26–37.

Yuan Y, Das SK, Li M. Vitamin D ameliorates impaired wound healing in streptozotocin-induced diabetic mice by suppressing NFκB-mediated inflammatory genes. *Biosci Rep* 2018;38:3.

doi:10.1042/BSR20171294. [Epub ahead of print: 27 Apr 2018].

Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetic nephropathy in diabetic mice. *J Am Soc Nephrol* 2018;103:322–35.

Botusan IR, Zheng X, Narayanan S, et al. Effects of alloxan on nephropathy in diabetic mice. *J Mol Med* 2017;95:1403–14.

Kuroda M, Kida K, Saito Y, et al. Anti-inflammatory action of naphthoquinone derivatives and their metabolite. *Biosci Rep* 2018;38:3.

doi:10.1042/BSR20180251. [Epub ahead of print: 27 Apr 2018].

Yuan Y, Das SK, Li M. Vitamin D ameliorates impaired wound healing in streptozotocin-induced diabetic mice by suppressing NFκB-mediated inflammatory genes. *Biosci Rep* 2018;38:3.

doi:10.1042/BSR20171294. [Epub ahead of print: 27 Apr 2018].

Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetic nephropathy in diabetic mice. *J Am Soc Nephrol* 2018;103:322–35.

Breyer MD, Böttiger E, Brosius FC, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol* 2005;16:27–45.

Burra N, Farahmand A, Kralisch S, et al. Calcium/calmodulin-dependent protein kinase II activation promotes angiogenesis in diabetic wounds by protecting endothelial cells against oxidative stress. *Arch Biochem Biophys* 2019;681:117–24.

Yang C-T, Chen L, Chen W-L, et al. Hydrogen sulfide primes diabetic wound to close through inhibition of Nrf2. *Mol Cell Endocrinol* 2019;480:74–82.

Galiano RD, Michaels J, Dobransky M, et al. Quantitative and reproducible murine model of excisional wound healing. *Wound Repair Regen* 2004;12:485–92.

Lundgren JM, Yu F, Gerasimenko JR. Splinting strategies to overcome confounding wound contraction in experimental animal models. *Adv Wound Care* 2013;2:142–8.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32 Suppl 1:166–76.

Lenzen S, The role of calprotectin- and streptozotocin-induced diabetes. *Diabetologia* 2008;51:216–26.

Breitkopf MR, Böttiger E, Brosius FC, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol* 2005;16:27–45.

Burra N, Farahmand A, Kralisch S, et al. Calcium/calmodulin-dependent protein kinase II activation promotes angiogenesis in diabetic wounds by protecting endothelial cells against oxidative stress. *Arch Biochem Biophys* 2019;681:117–24.

Yang C-T, Chen L, Chen W-L, et al. Hydrogen sulfide primes diabetic wound to close through inhibition of Nrf2. *Mol Cell Endocrinol* 2019;480:74–82.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32 Suppl 1:166–76.

Lenzen S, The role of calprotectin- and streptozotocin-induced diabetes. *Diabetologia* 2008;51:216–26.

Breyer MD, Böttiger E, Brosius FC, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol* 2005;16:27–45.

Burra N, Farahmand A, Kralisch S, et al. Calcium/calmodulin-dependent protein kinase II activation promotes angiogenesis in diabetic wounds by protecting endothelial cells against oxidative stress. *Arch Biochem Biophys* 2019;681:117–24.

Yang C-T, Chen L, Chen W-L, et al. Hydrogen sulfide primes diabetic wound to close through inhibition of Nrf2. *Mol Cell Endocrinol* 2019;480:74–82.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32 Suppl 1:166–76.

Lenzen S, The role of calprotectin- and streptozotocin-induced diabetes. *Diabetologia* 2008;51:216–26.

Breyer MD, Böttiger E, Brosius FC, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol* 2005;16:27–45.

Burra N, Farahmand A, Kralisch S, et al. Calcium/calmodulin-dependent protein kinase II activation promotes angiogenesis in diabetic wounds by protecting endothelial cells against oxidative stress. *Arch Biochem Biophys* 2019;681:117–24.

Yang C-T, Chen L, Chen W-L, et al. Hydrogen sulfide primes diabetic wound to close through inhibition of Nrf2. *Mol Cell Endocrinol* 2019;480:74–82.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32 Suppl 1:166–76.

Lenzen S, The role of calprotectin- and streptozotocin-induced diabetes. *Diabetologia* 2008;51:216–26.

Breyer MD, Böttiger E, Brosius FC, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol* 2005;16:27–45.

Burra N, Farahmand A, Kralisch S, et al. Calcium/calmodulin-dependent protein kinase II activation promotes angiogenesis in diabetic wounds by protecting endothelial cells against oxidative stress. *Arch Biochem Biophys* 2019;681:117–24.

Yang C-T, Chen L, Chen W-L, et al. Hydrogen sulfide primes diabetic wound to close through inhibition of Nrf2. *Mol Cell Endocrinol* 2019;480:74–82.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32 Suppl 1:166–76.