Effects of Experimental Diabetes, Uremia, and Malnutrition on Wound Healing

D. K. YUE, S. McLENNAN, M. MARSH, Y. W. MAI, J. SPALIVIERO, L. DELBRIDGE, T. REEVE, AND J. R. TURTLE

SUMMARY

The strength of linear wounds was studied in normal and diabetic rats in the first 8 wk after wounding. The strength of wounds from diabetic animals was found to be reduced compared with normal controls but could be improved by insulin treatment, especially when excellent metabolic control was achieved. There appeared to be both quantitative and qualitative defects in the formation of wound tissues in diabetic animals, because wound strength was not normalized when the thinner skin of diabetic animals was taken into consideration. This was different from the findings in rats with renal failure or malnutrition: in these two conditions, wound strength appeared reduced but was normalized when adjusted for skin thickness. Increased activity of aldose reductase did not appear to be an important factor in the impairment of wound healing in diabetes, because wound strength was not corrected by treatment with sorbinil, an aldose reductase inhibitor. The precise mechanism of abnormal wound strength in diabetes remains to be studied further, but careful control of diabetes, maintenance of nutrition, and treatment of systemic illness are important factors in the promotion of wound healing.
***Diabetes* 36:295–99, 1987**

Abnormal wound healing is a recognized phenomenon in diabetes and may lead to postoperative complications (1–4). To overcome this problem, it is widely accepted that diabetes should be brought under control as much as possible during the perioperative period. However, the precise relationship between metabolic control and wound strength is not known. It is therefore uncertain how vigorously hyperglycemia needs to be controlled to promote wound healing. We studied diabetic rats to evaluate the healing of linear surgical wounds and its relationship to degree of diabetic control. To elucidate the mechanism of abnormal wound healing in diabetes, we also examined the process of wound healing in uremic and in food-restricted rats to compare the effect of diabetes with that of systemic illness or malnutrition. Because increased aldose reductase activity has also been demonstrated to be a cause of vascular abnormalities in the granulation tissue of diabetic animals (5), we investigated the efficacy of the aldose reductase inhibitor sorbinil in improving wound healing in diabetes.

From the Departments of Medicine, Surgery, and Mechanical Engineering, The University of Sydney, and the Department of Endrocrinology, Royal Prince Alfred Hospital, Sydney, NSW, Australia.
Address correspondence and reprint requests to Dr. D. K. Yue, Department of Medicine, The University of Sydney, Sydney, NSW 2006, Australia.
Received for publication 18 February 1986 and accepted in revised form 3 September 1986.

MATERIALS AND METHODS

Animals and induction of diabetes. Female Wistar rats (150–200 g body wt) were obtained from the animal house of The University of Sydney. Diabetes was induced by injection of streptozocin (65 mg/kg i.v.); uninjected normal rats were used as controls. Diabetes was confirmed by the measurement of tail blood glucose levels on the 3rd day after injection of streptozocin; only animals with levels >22 mM were included in the study. Animals were supplied in batches of 30–40, and in some experiments two batches of well-matched animals with identical control groups were used and results were pooled. All procedures were approved by the Animal Care Ethics Review Committee of The University of Sydney.

Production of linear surgical wound. Three days after streptozocin injection, when diabetes was confirmed, animals were anesthetized with ketamine (10 mg/100 g i.p.; Parke-Davis, Caringbah, NSW, Australia). Two linear surgical wounds 4 cm long were produced in each rat by incising the skin perpendicular to the lumbar spine down to but not including the panniculus carnosus. The wounds were closed immediately with discontinuous silk sutures at 1-cm intervals and dusted with antibiotic powder containing bacitracin and neomycin (Wellcome Australia, Cabarita, NSW, Australia). Each animal also received one injection of penicillin (Vetspen

Plus, Glaxovet, Boronia, Victoria, Australia). Except for the time-course experiment, the wounds were allowed to heal for 8 wk, when the animals were killed with an overdose of intraperitoneal ketamine.

Blood was obtained by cardiac puncture for glycosylated hemoglobin and blood glucose determinations. The entire skin excluding the head and leg area was excised, fat and muscle were removed by gentle scraping, and the skin was flattened between two layers of foil, frozen in liquid nitrogen, and stored at −80°C until wound strength was measured.

Measurement of wound strength. The frozen skin was cut into four rectangular strips (1 cm wide, 4 cm long) perpendicular to the wound. The thickness of each piece of skin at the site of the wound was measured with calipers, and the skin was thawed to room temperature. The skin was secured in the clamps of the Instron 1195 testing machine (Instron Pty., Crows Nest, NSW, Australia) exactly 1 cm away from and parallel to the wound, giving a total distance of 2 cm between the clamps with the wound at the center. The Instron machine was set to a full scale load of 100 N, and a load-deflection curve of the skin was recorded. The crosshead speed was kept constant at 50 mm/min. Because wounded skin represents a nonhomogeneous tissue, it was not possible to evaluate the conventional stress versus strain relationship. The breaking strength of the wound was measured by the maximum load in N at the breaking point (6). To account for the variable skin thickness the results were also expressed as tensile strength defined as breaking strength in N/cross-sectional area of the wound (6). Because the strips were always 1 cm wide, the only variable in the calculation of cross-sectional area was the thickness of the skin. All strips obtained from the same experiment were measured within a few days by one observer who was not aware of the treatment status of the animals. The mean coefficient of variation of the measurement of different strips from each animal was 25%. To reduce variation, four pieces of skin from each animal (two central strips from each side) were studied, and the mean was taken as the breaking strength and tensile strength of that animal.

Effects of diabetes on wound healing. After induction of wounds, small groups of normal and diabetic animals were killed at 2, 3, 4, 6, and 8 wk, and wound strength was studied. After establishing that wound strength of normal and diabetic animals was significantly different at 8 wk, more normal and diabetic animals were studied at 8 wk.

Effects of insulin treatment on wound strength. Thirty-three rats were divided randomly into four groups [normal controls, diabetics receiving 9 U lente insulin (Novo, Copenhagen, Denmark) daily, diabetics receiving 3 U lente insulin daily, and untreated diabetic animals]. Linear wounds were induced as usual 3 days after streptozocin injection; after 8 wk the animals were killed, and the wound strength was measured.

Effects of uremia and food restriction. Rats with renal failure were produced as described previously by an ~65% nephrectomy carried out as a two-stage procedure (7). These rats had plasma creatinine levels ~2–3 times those of normal within a few days of the procedure. Linear wounds were induced as described, 3 days after the second stage of nephrectomy. In another group of animals, the rats were restricted to 50% normal food intake (10 g/day) from the time of wounding. In our control experiments, these rats lost weight slightly faster than diabetic animals. After the wounds had been present for 8 wk, the animals were killed, and skin was obtained for measurement of wound strength as previously described.

Effects of sorbinil treatment. In this experiment, diabetes and linear wounds were induced in the usual manner. Immediately afterward, half of the diabetic animals began sorbinil treatment by intragastric feeding at a dose of 5 mg/kg body wt; the other half remained untreated. A normal control group was studied in parallel, and after 8 wk the animals were killed, and wound strength was measured. The dosage of sorbinil chosen was one previously found to normalize tissue sorbitol levels (8,9).

Glycosylated hemoglobin measurement. Glycosylated hemoglobin levels were measured by a method of *m*-aminophenylboronic acid affinity chromatography described previously (10).

Statistical methods. All data are expressed as means ± SD, and results of different groups of animals were evaluated by multivariate analysis and analysis of variance with posthoc comparison by Duncan's multiple-range test (11). Curve fit-

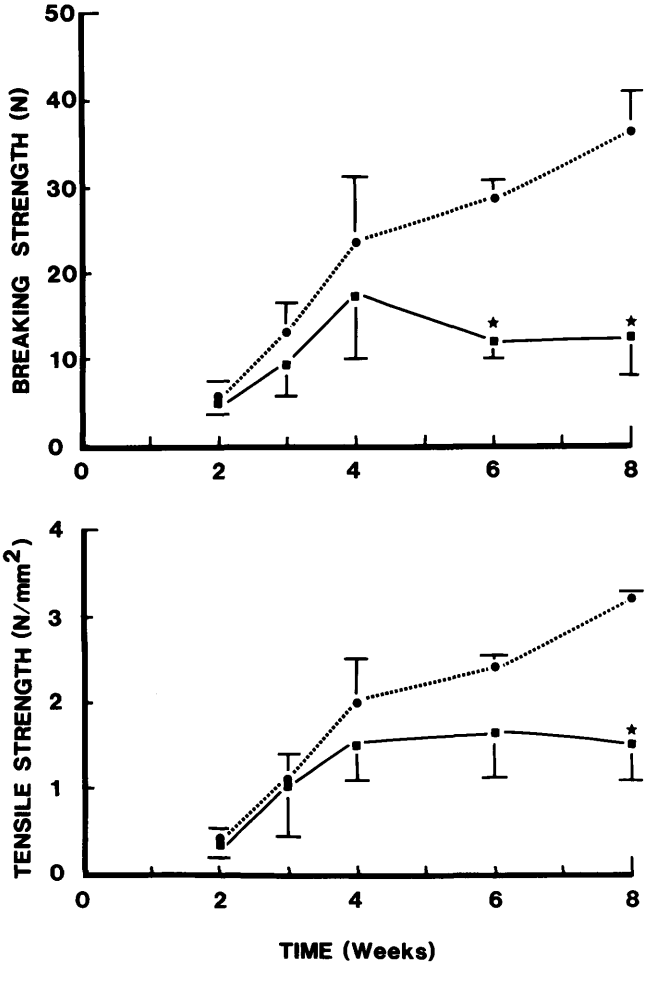

FIG. 1. Time course for increase in breaking strength and tensile strength of linear wounds. *Dashed line*, normal; *solid line*, diabetics. *N* = 4–6 animals at each time point. * Different from normal controls ($P < .01$).

TABLE 1
Effects of diabetes on wound strength

Group	N	Body wt* (g)	Plasma glucose* (mg/dl)	Glycosylated hemoglobin* (%)	Breaking strength (N)	Tensile strength (N/mm²)
Normal	11	205 ± 9	5.2 ± 0.3	9.5 ± 3.6	34.0 ± 5.0	3.1 ± 0.4
Untreated diabetic	9	163 ± 10	30.2 ± 4.3†	19.6 ± 2.8†	13.7 ± 4.3†	1.8 ± 0.5†

* Taken when killed.
† Different from normal controls ($P < .05$).

ting was performed by polynomial regression and correlation was calculated by the least-squares method (12).

RESULTS

Wound strength of normal and diabetic animals. The increases in the strength of wounds of normal and diabetic animals are shown in Fig. 1. The breaking strength of wounds from diabetic animals increased at a slower rate than that of normal controls, and the difference between the normal and diabetic animals became progressively greater over the 8-wk period. This pattern was still observed when the results were adjusted for the cross-sectional area of the skin and expressed as tensile strength, although the differences between normal and diabetic animals were smaller in magnitude. The differences in the breaking strength and tensile strength of the normal and diabetic animals were statistically significant at 8 wk. The results of a larger group of animals studied at 8 wk are shown in Table 1.

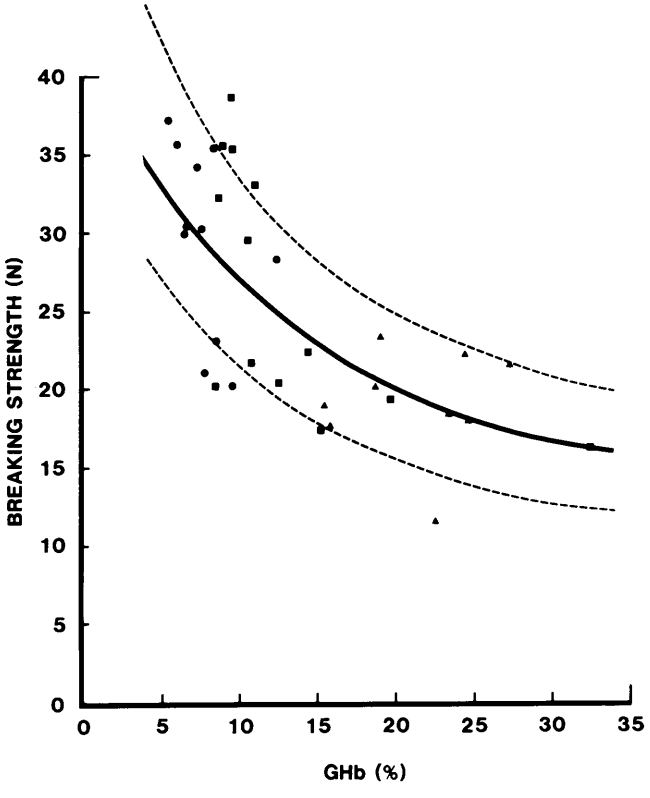

FIG. 2. Relationship between breaking strength of wounds and glycosylated hemoglobin levels. ●, Normal; ▲, diabetics; ■, insulin-treated diabetics. *Solid line*, line of best fit described by equation $y = 66.37x^{-0.39}$; *dotted lines*, ±1SD.

Effects of diabetic control on wound strength. The relationship between breaking strength (y) and glycosylated hemoglobin levels (x) was found to fit best a power-law relationship described by the equation $y = 66.37x^{-0.39}$, with $r = .63$ (Fig. 2). The dashed curves represent ±1SD from the mean curve, and it can be seen that almost all the experimental data are bound by these two curves. A similar power-law relationship also held when wound strength was expressed as tensile strength ($y = 6.0x^{-0.34}$, $r = .63$). Multivariate analysis showed that diabetic control was the major determinant of wound strength, whereas the associated weight loss had no independent effect on either breaking strength or tensile strength (breaking strength $F_{1,39} = 0.61$, $P = .44$; tensile strength $F_{1,39} = 0.30$, $P = .59$). Insulin dose dependently exerted its effect on breaking strength and tensile strength (breaking strength $F_{1,40} = 241.1$, $P < .0001$, tensile strength $F_{1,40} = 258.9$, $P < .00001$).

Effects of uremia or food restriction on wound strength. The effects of uremia and food restriction on body weight, biochemical data, and wound strength are shown in Table 2. Both uremia and food restriction resulted in a reduction in the mean breaking strength of wounds. However, in contrast to diabetic animals, the mean tensile strength of the wounds was not different from that of normal controls after adjustment for the cross-sectional area of the skin.

Effects of sorbinil treatment on wound strength. The treatment of diabetic animals with sorbinil had no effect on the reduced wound strength, which remained significantly below that of control animals and not different from untreated diabetic animals. Results are summarized in Table 3.

DISCUSSION

Impairment of wound healing is a commonly encountered postoperative complication in diabetes (1–4). It is widely accepted that good perioperative control of diabetes is desirable to promote wound healing. However, this treatment strategy is often not implemented vigorously because of practical considerations such as lack of hospital beds for stabilization of diabetes, desire to minimize costs, and difficulty of obtaining rapid blood glucose determinations. There is also a lack of enthusiasm on the part of health professionals to stress the importance of achieving good control, partly due to a lack of objective evidence that clinical outcome depends on such measures and because it is not known what degree of control is necessary to achieve beneficial results. Goodson and Hunt (1,3) had previously demonstrated beneficial effects of insulin on wound healing, but

TABLE 2
Effects of uremia and food restriction on wound strength

Group	N	Wt* (g)	Plasma creatinine (μM)	Breaking strength (N)	Tensile strength (N/mm²)
Normal	8	216 ± 18	40 ± 8.0	33.7 ± 5.7	3.3 ± 0.5
Uremic	7	208 ± 13	131 ± 42.0†	25.5 ± 7.9†	2.9 ± 0.9
Food restricted	9	154 ± 13	38 ± 4.0	25.9 ± 5.8†	2.7 ± 0.4

* Taken when killed.
† Significantly different from normal controls ($P < .05$).

this was done before the degree of diabetic control could be accurately quantified by measurement of glycosylated hemoglobin. This study clearly established that diabetic wounds were weaker than normal and was terminated at 8 wk after wounding, when the strength of wounds in normal animals is ~50% of normal unwounded skin (unpublished data). It is possible that differences in normal and diabetic animals would be even greater if the studies had been extended further. We limited our studies to 8 wk because this is the duration relevant to wound healing in most clinical settings. The nonlinear relationship between wound strength and glycosylated hemoglobin levels found in this study indicates that the process of healing is not a simple function of the overall diabetic control achieved; rather, near normalization of blood glucose levels is necessary before significant improvement in wound strength occurs, suggesting the importance of meticulous diabetic control during the perioperative period.

Diabetic animals are invariably sick, malnourished, and have lost considerable weight as well as being hyperglycemic. These factors are interrelated, and it is not certain that the high blood glucose level is causing the abnormalities in wound strength. It is therefore necessary to study other animal models with systemic illness or malnutrition to answer this question. Rats with uremia or food restriction were selected for this purpose. When the results were analyzed by multivariate analysis, the weight of the animals was not a significant factor in determining wound strength, suggesting that weight loss was not the cause of impaired wound healing in diabetes. Similarly, malnutrition was more important than weight loss as the underlying mechanism of healing abnormalities during food restriction. In this regard, vitamin A and C deficiency may play a role in our results, because both can be important in wound healing (2).

Wound strength in diabetic animals was found to be reduced even when the thinner skin of these animals was taken into account. Thus, the observed weakness of the wound cannot be explained solely on the basis of a reduced skin thickness of these animals. In this respect, diabetes behaved differently from uremia and malnutrition. In the two latter conditions, although the wounds were weak in absolute terms, they were of normal strength when skin thickness was taken into consideration. These results suggest that in diabetes there are both quantitative and qualitative defects in the formation of wound tissue, whereas in uremia and malnutrition the defect is primarily quantitative due to thinning of the skin.

TABLE 3
Effects of sorbinil treatment on wound strength

Group	N	Breaking strength (N)	Tensile strength (N/mm²)
Normal	4	45.0 ± 11.3	3.7 ± 0.6
Diabetic	10	14.7 ± 7.7*	1.8 ± 0.7*
Sorbinil-treated diabetic	10	13.5 ± 5.0*	1.6 ± 0.4*

* Significantly different from normal controls ($P < .01$).

This has an interesting parallel with the results we reported in a study of granulation tissue formation in diabetes, uremia, and malnutrition (7). In all three conditions, the mass of granulation tissue recovered from wound cylinders implanted subcutaneously in rats was reduced, but only in diabetes was a decrease in collagen concentration of the granulation tissue observed. The deficient collagen content of granulation tissue may be one of the qualitative differences between diabetic and normal wound tissues. However, to prove this hypothesis it is necessary to quantitate accurately the amount of granulation tissue and collagen present in the actual wound without contamination of adjacent tissue. This is a difficult task; a previous study with radioactive proline as a marker showed that the active part of a linear surgical wound is only 1–2 mm wide (13). Our measurement of skin thickness at the wound site may be a poor guide of the amount of wound tissue present, and it does not give an indication of its chemical composition.

Even if a reduction in granulation tissue mass and its collagen concentration can be shown to be present at the wound site, other factors are probably playing a role in the defective wound strength of diabetic animals. Previous studies with normal animals had indicated that wound strength continued to increase between the 3rd and 7th wk after wounding, whereas collagen deposition had already reached a plateau by this stage (14). This increase in strength is generally attributed to progressive turnover with remodeling and cross-linking of the collagen fibers (14,15). The progressively larger differences between the wound strength of normal and diabetic animals between the 4th and 8th wk of diabetes in this study would suggest that these aspects of wound maturation in diabetes are abnormal. The mechanism for this is intriguing because diabetes is well known to be associated with increased turnover and cross-linking of collagen, conditions associated with increased strength of collagen fibers.

Augmented biomechanical strength of unwounded skin and other collagen-containing tissues has been described (16–20). Thus the precise cause of impaired wound healing in diabetes is not clear, and the relative importance of diminished granulation tissue mass, reduced collagen concentration of the wound tissue, and defects in collagen ma-

turation remain to be defined. Although abnormal vascular permeability of granulation tissue has recently been demonstrated in diabetic animals and shown to respond to treatment with aldose reductase inhibitor (5), the wound healing in our studies was not normalized by treatment with sorbinil used in a dosage that was effective in normalizing polyol accumulations in diabetic and galactosemic rats (8,9). However, a higher dosage of sorbinil would need to be employed before it can be certain that aldose reductase plays little part in the process of wound healing.

The results of this study illustrate the importance of optimizing diabetic control as much as possible during the perioperative period because near normalization of healing requires very tight diabetic control. At the clinical level, treatment of other concurrent systemic illness and maintenance of good nutrition would also be complementary therapeutic modalities that assist the recovery of diabetic patients undergoing surgery. The fundamental basis of the wound abnormalities in diabetes remains an interesting problem for further investigation.

ACKNOWLEDGMENTS

This study was supported by the National Health and Medical Research Council of Australia and the Kellion Foundation of Australia.

REFERENCES

1. Goodson WH, Hunt TK: Wound healing and the diabetic patient. *Surg Gynecol Obstet* 149:600–608, 1979
2. Seifter E, Rettura G, Padawer J, Stratford F, Kambosos D, Levenson SM: Impaired wound healing in streptozotocin diabetes: prevention by supplemental vitamin A. *Ann Surg* 194:42–50, 1981
3. Goodson WH, Hunt TK: Studies of wound healing in experimental diabetes mellitus. *J Surg Res* 22:221–27, 1977
4. Prokash MK, Sharma LK: Studies in wound healing in experimental diabetes. *Int Surg* 59:25–28, 1974
5. Williamson JR, Chang K, Rowold E, Marvel J, Tomlinson M, Sherman WR, Ackermann KE, Kilo C: Sorbinil prevents diabetes-induced increases in vascular permeability but does not alter collagen cross-linking. *Diabetes* 34:703–705, 1985
6. Levenson SM, Geever EF, Crowley LV, Oates JF, Berard CW, Rosen H: The healing of rat skin wounds. *Ann Surg* 161:293–308, 1965
7. Yue DK, Swanson B, McLennan S, Marsh M, Spaliviero J, Delbridge L, Reeve T, Turtle JR: Abnormalities of granulation tissue and collagen formation in experimental diabetes, uremia and malnutrition. *Diabetic Med* 3:221–25, 1986
8. Yue DK, Hanwell MA, Satchell PM, Turtle JR: The effect of aldose reductase inhibition on motor nerve conduction velocity in diabetic rats. *Diabetes* 31:789–94, 1982
9. Yue DK, Hanwell M, Satchell P, Turtle JR: The effects of aldose reductase inhibitor on nerve sorbitol and myoinositol concentrations in diabetic and galactosemic rats. *Metabolism* 33:1119–22, 1984
10. Yue DK, McLennan S, Church DB, Turtle JR: The measurement of glycosylated hemoglobin in man and animals by aminophenylboronic acid affinity chromatography. *Diabetes* 31:701–705, 1982
11. Dixon WJ, Brown MB: *BMDP Biomedical Computer Programs, P-Series*. Berkeley, Univ. of California Press, 1979
12. Kuo, S: *Fortran Program for Least Squares Polynomial Fit. Computer Application of Numerical Methods*. Menlo Park, CA, Addison-Wesley, 1972
13. Bevin AG, Madden JW: Localization of collagen synthesis in healing wounds. *Surg Forum* 20:65–66, 1969
14. Madden JW, Peacock EE: Studies on the biology of collagen during wound healing. III. Dynamic metabolism of scar collagen and remodeling of dermal wounds. *Ann Surg* 174:511–18, 1971
15. Danielsen CC, Fogdestom I: Delayed primary closure: collagen synthesis and content in healing rat skin incisions. *J Surg Res* 31:210–17, 1981
16. Yue DK, McLennan S, Delbridge L, Handelsman DJ, Reeve T, Turtle JR: The thermal stability of collagen in diabetic rats: correlation with severity of diabetes and non-enzymatic glycosylation *Diabetologia* 24:282–86, 1983
17. Enser M, Avery NC: Mechanical and chemical properties of the skin and its collagen from lean and obese-hyperglycaemic (*ob/ob*) mice. *Diabetologia* 27:44–49, 1984
18. Andreassen TT, Seyer-Hansen K, Oxland H: Biochemical changes in connective tissue induced by experimental diabetes. *Acta Endocrinol* 98:432–36, 1981
19. Chang K, Uitto, J, Rowold EA, Grant GA, Kilo C, Williamson JR: Increased collagen cross-linkages in experimental diabetes: reversal by β-aminoproprionitrile and D-penicillamine. *Diabetes* 29:778–81, 1980
20. Schneir M, Bowersox J, Ramamurthy N, Yavelon J, Murray J, Edlin-Folz E, Golub L: Response of rat connective tissues to streptozotocin-diabetes. *Biochim Biophys Acta* 583:95–102, 1979