ORIGINAL ARTICLE

Decrease of Collagen Deposition in Wound Repair in Type 1 Diabetes Independent of Glycemic Control

Eva Black, MD; Jette Vibe-Petersen, MD; Lars N. Jorgensen, MD; Søren M. Madsen, PhD; Magnūs S. Ågren, DMSc; Per E. Holstein, MD, DMSc; Hans Perrild, MD; Finn Gottrup, MD, DMSc

Hypothesis: Type 1 and type 2 diabetes mellitus and glycemic control influence wound healing in humans.

Design: Experimental study using a human wound-healing model.

Setting: Collaboration among a multidisciplinary wound-healing department, department of medicine, and research laboratories.

Patients, Control Subjects, and Methods: In 34 patients with type 1 (insulin-dependent) and 25 with type 2 (non–insulin-dependent) diabetes and 5 nondiabetic control subjects matched with the type 2 diabetic patients, wound-healing capacity was determined as subcutaneous accumulation of collagen measured as hydroxyproline. Two expanded polytetrafluoroethylene tubes were implanted and removed 10 days later. The hydroxyproline level was determined by means of high-performance liquid chromatography; the collagenase activity, by using a radiolabeled collagen substrate. Proliferation of fibroblasts cultured from the wounds was studied in patient groups.

Results: The deposition of hydroxyproline decreased by 40% ($P = .03$) in type 1 compared with type 2 diabetes (median, 0.70 vs 1.16 nmol/mg; interquartile range, 0.48-1.04 vs 0.56-1.63 nmol/mg), which in turn did not differ significantly from that of controls (median, 1.35 nmol/mg; interquartile range, 0.72-1.88 nmol/mg). The decreased collagen deposition in type 1 diabetes was not caused by increased collagenase activity. The deposition of hydroxyproline did not correlate significantly ($r_s = 0.07$; $P = .63$) with glycosylated hemoglobin levels in either diabetic group. Fibroblast growth was also decreased in type 1 compared with type 2 diabetic patients and controls.

Conclusions: Collagen deposition in acute wounds is impaired in type 1 diabetes, possibly due to a decreased fibroblast proliferation. In type 2 diabetes, collagen deposition is normal. Glycemic control does not influence collagen deposition in acute wound repair in type 1 or in type 2 diabetes mellitus.

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From the Copenhagen Wound Healing Center (Drs Black, Jorgensen, Holstein, and Gottrup) and the Medical Department I, Bispebjerg Hospital, University of Copenhagen (Drs Vibe-Petersen and Perrild), Copenhagen, Denmark; and Coloplast Research (Dr Madsen) and Aagren Dermaconsulting ApS (Dr Ågren), Humlebæk, Denmark.

IN DIABETIC foot ulcers, healing is severely impaired.[1-3] It is generally assumed that glycemic control is important in optimizing wound healing. A number of animal experiments and in vitro human studies support this assumption. Experimental diabetes is associated with an impaired inflammatory response, reduced deposition of collagen, and decreased mechanical strength of incisional cutaneous or intestinal wounds, and healing is almost normalized with insulin treatment.[4-8] In human studies, fibroblasts from healthy human skin have been shown to proliferate slower when cultured in high glucose levels and to acquire resistance to insulin and growth factors.[9] Fibroblasts cultured from chronic wounds demonstrate impaired proliferation, in particular from diabetic foot ulcers.[10]

However, limited and inconsistent information is available on the influence on wound healing in vivo in humans with type 1 and type 2 diabetes and on the influence of glycemic control. An uncontrolled clinical study suggested that improved metabolic control improves wound healing.[11] Capillary microscopy of the toes in diabetic patients has shown pronounced capillary ischemia in patients with poor metabolic control,[12] and insulin infusion improves capillary blood flow in the skin of the foot in diabetic patients.[13] However, diabetes does not appear to be a risk factor for postoperative wound complications in major surgery.[14-16] Moreover, diabetic patients with postoperative complications were found to have significantly lower blood glucose levels, ie, closer to normoglycemia preoperatively and postoperatively, than those without complications.[17] Finally, collagen accumulation in acute wounds in men with well-controlled type 1 diabetes was similar to that of nondiabetic subjects.[18]

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Table 1. Baseline Characteristics for Patients With Types 1 and 2 Diabetes*

	Patient Groups			
	Type 1 Diabetes (n = 34)	Type 2 Diabetes (n = 25)	Difference (95% Confidence Limits)	*P* Value†
No. of male/female	28/6	22/3	−11% (−44% to 23%)	.83
Age, y	40.5 (28.8 to 50.0)	60.0 (52.5 to 69.0)	−19.5 (−25.4 to −13.6)	<.001
Duration of diabetes, y	10.5 (5.8 to 14.0)	10.0 (4.0 to 16.5)	0.5 (−4.4 to 5.3)	.84
BMI	23.5 (21.4 to 26.1)	29.1 (26.9 to 32.1)	−5.43 (−7.71 to −3.15)	<.001
No. with retinopathy/no retinopathy	10/24	11/14	−15% (−39% to 10%)	.38
No. of smokers/nonsmokers	17/17	12/13	2% (−23% to 27%)	>.99
Daily alcohol consumption, U‡	1.0 (1.0 to 1.3)	1.0 (0.0 to 3.0)	−0.2 (−1.1 to 0.5)	.99
Systolic blood pressure, mm Hg	140 (134 to 150)	160 (133 to 170)	−12 (−23 to −4)	.03
Diastolic blood pressure, mm Hg	75 (70 to 85)	85 (80 to 90)	−5 (−10 to 0)	.02
Fasting C peptide, ng/mL	<0.300 (<0.300 to 0.513)	1.97 (1.56 to 2.53)	−1.85 (−2.29 to −1.41)	<.001
HbA_{1c}, %	8.3 (7.5 to 9.4)	9.4 (8.0 to 10.8)	−0.8 (−1.7 to 0.1)	.08
Serum hemoglobin, g/dL	15.0 (14.0 to 15.1)	15.0 (14.1 to 15.6)	−0.2 (−0.6 to 0.3)	.79
Platelets, $\times 10^3$/µL	252 (195 to 280)	192 (167 to 230)	38 (11 to 64)	.006
Leukocytes, cells/µL	6100 (5200 to 7200)	7300 (6400 to 8000)	−100 (−1100 to 900)	.42
Neutrophils, cells/µL	3500 (3000 to 4600)	4500 (3300 to 5100)	0 (−700 to 800)	.52
C-reactive protein, mmol/L	<35 (<35 to <35)	38 (<35 to 88)	33 (−96 to 162)	.01
Sedimentation rate, mm/h	4 (2 to 8)	12 (6 to 19)	−7 (−11 to −2)	.003
Serum creatinine, mg/dL	0.98 (0.93 to 1.03)	1.03 (0.93 to 1.10)	−0.05 (−0.11 to 0.03)	.14
Serum transferrin, mg/dL	266 (242 to 290)	347 (323 to 363)	73 (−97 to −48)	<.001
Serum albumin, g/dL	4.12 (3.84 to 4.22)	3.98 (3.85 to 4.18)	0 (−0.14 to 0.14)	.23
Coagulation factors II, VII, and X, %	92 (82 to 100)	116 (85 to 127)	−15 (−26 to −5)	.001
Total cholesterol, mg/dL	178 (135 to 205)	208 (197 to 232)	−46 (−66 to −23)	.001
LDL cholesterol, mg/dL	100 (77 to 120)	127 (120 to 158)	−35 (−50 to −15)	.002
HDL cholesterol, mg/dL	50 (42 to 62)	54.1 (46 to 58)	8 (−4 to 15)	.79
Fasting serum triglycerides, mg/dL	81 (65 to 119)	138 (97 to 190)	−115 (−204 to −27)	.001

Abbreviations: BMI, body mass index (calculated by dividing weight in kilograms by the square of height in meters); HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI conversion factors: To convert C peptide to nanomoles per liter, multiply by 0.333; hemoglobin to millimoles per liter, by 0.6206; creatinine to micromoles per liter, by 88.4; total, LDL, and HDL cholesterol to millimoles per liter, by 0.0259; triglycerides to millimoles per liter, by 0.0113.

*Unless otherwise indicated, data are given as median (interquartile range).

†Determined by Mann-Whitney or Fisher exact test.

‡One unit of alcohol is approximately 16 g.

The present investigation was undertaken to elucidate the influence of types 1 and 2 diabetes mellitus and control of glycemia on the wound-healing potential in human tissue not compromised by decreased perfusion, infection, or neuropathy. Wound healing was evaluated using a standardized model in which granulation tissue is formed within tubes made of expanded polytetrafluoroethylene (ePTFE) implanted subcutaneously. The wound-healing response was quantified as the amount of collagen deposited in the tubes. In addition, fibroblasts cultured from the granulation tissue were compared with respect to proliferation.

METHODS

PATIENTS

Fifty-nine patients received a diagnosis of type 1 (insulin-dependent) or type 2 (non–insulin-dependent) diabetes mellitus according to the World Health Organization classification.[19] Thirty-four patients were classified as having type 1 and 25, as having type 2 diabetes. Baseline characteristics are shown in **Table 1**. None of the patients had chronic ulceration, peripheral neuropathy (measured by means of biothesiometry), peripheral arterial disease (evaluated by means of foot pulsations and/or systolic blood pressure measurements on the ankle and first toe), macroalbuminuria (defined as 24-hour urine albumin excretion exceeding 300 mg), infection, or a hepatologic or hematologic disorder (judged by means of laboratory tests and medical history). Retinopathy was frequent in patients with types 1 and 2 diabetes (Table 1). No pregnant or breastfeeding women participated. All patients were recruited from the endocrinological outpatient clinic at our institution. Patients received standard diabetic treatment during the study period.

In addition, 5 healthy volunteers were recruited as control subjects and 10 patients from each diabetic group were matched with the controls by age, sex, and smoking habits (**Table 2**).

The study was performed in accordance with the Helsinki Declaration II and approved by the Copenhagen Ethics Committee, Copenhagen, Denmark. All participants were included after giving their written informed consent.

WOUND MODEL

Two ePTFE tubes (Impra; International Polymer Engineering Inc, Tempe, Ariz), 8 cm in length, with an inner diameter of 0.12 cm, an outer diameter of 0.24 cm, and a pore size of 90 to 120 µm, were inserted subcutaneously in the lateral part of the upper arm during local anesthesia with 20 mL of subcutaneous 1% mepivacaine hydrochloride (Carbocain; AstraZeneca Pharmaceuticals LP, Södertälje, Sweden). The ePTFE implants were placed parallel in a distal-proximal direction, with a distance of 4 cm. The technique of insertion using a 0.25-cm cannula has been described previously.[20] All patients received

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Table 2. Baseline Characteristics for Controls and Matched Groups of Diabetic Patients*				
		Matched Patient Groups		
	Healthy Controls (n = 5)	Type 1 Diabetes (n = 10)	Type 2 Diabetes (n = 10)	*P* Value†
No. of male/female	4/1	9/1	9/1	.83
Age, y	51 (50 to 57)	51 (47 to 59)	56 (51 to 61)	.41
Duration of diabetes, y	13.5 (5.8 to 25.5)	8.5 (4.8 to 12.5)	6.2 (−1.2 to 13.6)	.15
BMI	32.3 (31.3 to 35.0)	21.4 (20.2 to 26.0)	28.2 (26.6 to 32.7)	<.001
No. with retinopathy/no retinopathy	. . .	5/5	3/7	.45
No. of smokers/nonsmokers	3/2	6/4	7/3	.88
Daily alcohol consumption, U‡	2 (1.5 to 4.5)	1 (1 to 3.5)	1.5 (0 to 3.8)	.59
Fasting C peptide, ng/mL	2.84 (2.22 to 3.35)	<0.30 (<0.30 to 0.31)	2.18 (1.65 to 2.51)	<.001
HbA_{1c}, %	6.0 (5.3 to 6.7)	8.8 (7.8 to 9.8)	9.1 (7.7 to 11.3)	.009
Serum hemoglobin, g/dL	15.1 (14.1 to 16.3)	15.1 (14.7 to 16.0)	15.1 (14.8 to 15.5)	>.99
Platelets, $\times 10^3$/μL	242 (210 to 327)	248 (220 to 274)	235 (166 to 264)	.62
Leukocytes, cells/μL	8800 (7300 to 11 300)	6400 (5400 to 9100)	7000 (4900 to 8200)	.19
Neutrophils, cells/μL	5000 (4200 to 7200)	4000 (3000 to 6200)	3900 (3000 to 4800)	.22
C-reactive protein, mmol/L	65 (57 to 91)	<35	38 (<35 to 59)	.02
Sedimentation rate, mm/h	10 (6 to 22)	4 (2 to 10)	8 (4 to 18)	.16
Serum creatinine, mg/dL	0.97 (0.88 to 1.06)	1.01 (0.97 to 1.03)	0.97 (0.88 to 1.03)	.50
Serum albumin, g/dL	3.94 (3.89 to 4.21)	4.07 (3.87 to 4.22)	4.19 (4.01 to 4.40)	.27
Coagulation factors II, VII, and X, %	107 (94 to 130)	99 (94 to 100)	124 (112 to 130)	.009
Total cholesterol, mg/dL	255 (239 to 275)	193 (162 to 228)	220 (197 to 236)	.02
LDL cholesterol, mg/dL	170 (139 to 201)	108 (89 to 124)	124 (120 to 158)	.007
HDL cholesterol, mg/dL	46 (46 to 54)	58 (46 to 89)	50 (31 to 62)	.25
Fasting serum triglycerides, mg/dL	132 (96 to 312)	91 (71 to 151)	149 (101 to 245)	.13

Abbreviations are explained in the first footnote to Table 1.

SI conversion factors: To convert C peptide to nanomoles per liter, multiply by 0.333; hemoglobin to millimoles per liter, by 0.6206; creatinine to micromoles per liter, by 88.4; total, LDL, and HDL cholesterol to millimoles per liter, by 0.0259; triglycerides to millimoles per liter, by 0.0113.

*Unless otherwise indicated, data are given as median (interquartile range). Healthy controls had fasting blood glucose levels within the reference range. Abbreviations are explained in the first footnote to Table 1.

†Determined by means of Kruskal-Wallis and χ^2 tests.

‡One unit of alcohol is approximately 16 g.

1.5 g of intravanous cefuroxime sodium (Zinacef; GlaxoSmith-Kline, London, England) before the implantation of the ePTFE tubes.

DETERMINATION OF HYDROXYPROLINE LEVEL

The ePTFE tubes were removed 10 days after the implantation. Two sections of the middle part of each tube (each 15-20 mm long) were stored in acetone at 4°C, processed starting with delipidization in acetone and diethyl ether, and dried. The weight of the tube sections was then measured. The tubes were hydrolyzed in an oxygen-free atmosphere of hydrogen chloride for 24 hours at 114°C. The hydrolysis residues were reconstituted in 0.1M hydrochloric acid, and duplicate aliquots amounting to one tenth of the whole sample were taken into separate polypropylene tubes, to which 45 nm of the internal standard L-citrulline (Sigma-Aldrich Corp, Steinheim, Germany) was added. The dried mixtures were reacted with phenylisothiocyanate using a standard procedure.[21] The phenylthiocarbamoyl derivatives from the amino acids were analyzed in a high-performance liquid chromatography system (Waters Corporation, Milford, Mass) using a 3-μm, 150×4.6-mm column (Thermo Hypersil, Runcorn, England). The amino acid derivatives were eluted with a gradually increasing concentration from acetonitrile in aqueous 0.1M sodium acetate buffer with 4mM triethylamine (pH, 5.70). Absorbance of the eluate was monitored at 254 nm. Linear calibration curves based on areas under the peaks in chromatograms were constructed from standards of L-4-hydroxyproline (Merck KGaA, Darmstadt, Germany) and a high-purity amino acid calibration standard for protein hydrolyzates (Amino Acid Standard H; Pierce Biotechnology, Inc, Rockford, Ill) run in parallel to the unknown samples and representing the range from 0.5 to 200 nm. The limit of detection was 0.4 nm per sample, and the repeatability in determination was 1.8 percentage points. The hydroxyproline contents of each ePTFE tube were expressed in nanomoles per milligram of ePTFE tube with ingrown granulation tissue (dry weight).

COLLAGENASE ASSAY

To assay for collagenase activity, samples were selected from 8 patients with type 1 diabetes and 8 patients with type 2 diabetes to match the whole group of patients with type 1 diabetes (n=34) and the whole group of patients with type 2 diabetes (n-25), respectively. Matching variables were hydroxyproline levels, sex, age, diabetes duration, levels of hemoglobin, glycosylated hemoglobin (HbA_{1c}), and C peptide, and body mass index (BMI; calculated by dividing weight in kilograms by the square of height in meters). Two separate segments, each about 8 mm in length, from each of 2 frozen (−70°C) ePTFE tubes from each patient were weighed (mean weight of 1 segment, 23 mg); the length was measured with a caliper ruler. The ePTFE tubes were homogenized in a buffer consisting of 25mM Tris hydrochloride (pH, 7.5), 10mM calcium chloride, and 0.25% Triton X-100 (100 μL of buffer/mm of ePTFE tube)[22] and the extracts were pooled. Total collagenase activity was assayed using the method of Dean and Woessner[23] with minor modifications. The substrate, tritiated, acetylated, telopeptide-free rat-skin collagen (17.5 μg collagen, 4.4×10^6 cpm/mg), was used for each assay, in a final volume of 110 μL of extract and assay buffer. The assay buffer was composed of 0.2M sodium chloride, 0.01M calcium chloride, 0.03% (vol/vol) polyoxyethylene 23 lauryl ether (Brij 35; Sigma-Aldrich Corp), 0.02% sodium azide, and 0.05M Tris hydrochloride (pH, 7.5). Samples

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in duplicate were incubated for 18 hours at 30°C with 0.5mM aminophenylmercuric acetate present during the assay. At the end of this incubation, the already-cleaved collagen molecules were further digested using trypsin and α-chymotrypsin for 1.5 hours at 31.5°C. The undigested collagen was precipitated by adding an equal volume of 20% (wt/vol) trichloroacetic acid and centrifuging. Reduction and alkylation, followed by dialysis against the assay buffer did not increase activity of the extracts further.[24] Freezing extracts once at −70°C increased active collagenase to about 40% of total collagenase activity, whereas it was less than 5% without prior freezing. One unit of enzyme degrades collagen at the rate of 1 µg/min at 30°C. The activity of collagenase was expressed as milliunits per milligram (wet weight) of ePTFE tube with ingrown granulation tissue.

FIBROBLAST CULTURES

For this study, ePTFE implants were obtained from 4 patients with type 1 diabetes and 8 with type 2 diabetes selected at random among the matched patients and from 4 controls. Granulation tissue was explanted from about 10 mm of 2 separate ePTFE tubes from each patient. The ePTFE tubes were suspended in phosphate-buffered saline at 5°C before explanting within 4 hours after harvesting. First, the ePTFE tubes were immersed in thrombin (1.5 National Institutes of Health U/mL; 850-1; Sigma-Aldrich Corp, St Louis, Mo), dissolved in Dulbecco modified Eagle medium (DMEM) with 25mM HEPES, and minced (1 mm^3). The explants (5-10 explants per dish) were placed on a drop (5-10 µL) of fibrinogen (3 mg/mL; F-8630; Sigma-Aldrich Corp) containing 50-mg/mL plasmin inhibitor (Cyclokapron; Pharmacia & Upjohn Animal Health AB, Helsingborg, Sweden) in the DMEM-HEPES medium in a 60-mm Petri dish. The cell yield and growth ability were improved by using this method compared with placing explants directly on the plastic surface of the Petri dishes.[25] Fibroblast outgrowth was supported with fibroblast growth medium (composed of fibroblast basal medium supplemented with basic fibroblast growth factor [1 ng/mL], insulin [5 µg/mL], gentamicin [50 µg/mL], and amphotericin B [10 ng/mL]) (Clonetics Corp, San Diego, Calif) with 10% heat-inactivated, mycoplasma-screened fetal calf serum (approved by the European Community) (GIBCO BRL; Invitrogen Corp, Carlsbad, Calif). The same batch of serum was used throughout the study. The glucose concentration of the complete medium was 7.6mM. Cultures were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide to air. Culture medium was changed twice weekly. At 80% confluence, typically within 4 weeks after explanting, fibroblasts were subcultivated using a mycoplasma-tested combination of trypsin and EDTA (0.05%/0.02% in phosphate-buffered saline; pH, 7.4) and transferred to 175-cm^2 tissue-culture flasks. Cells were then split at a ratio of 1:3 at confluence. Cells of the third passage were used for the growth kinetics study, which occurred about 6 weeks after explanting. On day 0, 3.5×10^3 cells/cm^2 were seeded in 24-well plates in 1 mL of complete medium per well and were enumerated 3, 4, 5, 6, 7, 8, 9, and 10 days postplating in triplicates using a hemocytometer. Cells were refed with new medium on days 4 and 7 during the 10-day growth period.[25]

STATISTICS

We analyzed the data obtained by the Mann-Whitney (unpaired data) and Wilcoxon (paired data) tests. We tested correlations by the Spearman rank test, and differences between groups by the Kruskal-Wallis and Fisher exact tests. A multivariate analysis of variance was used to test for confounding factors. A 2-sided statistical significance level of 5% was used.

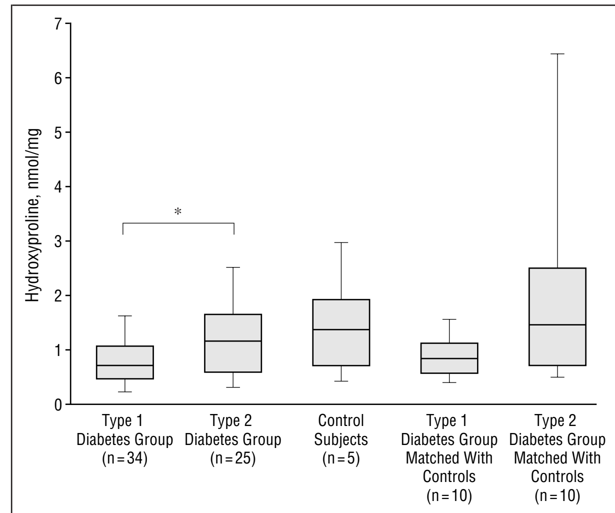

Figure 1. Box plots expressing median (horizontal line), interquartile range (shaded box), and the total range (limit lines) of deposition levels of hydroxyproline in granulation tissue. Patients with type 1 diabetes (whole group) deposited statistically significantly less hydroxyproline than those with type 2 diabetes (whole group). Asterisk indicates $P = .03$.

Unless otherwise indicated, data are presented as median values (interquartile ranges).

RESULTS

BASELINE VARIABLES

Compared with patients with type 1 diabetes, those with type 2 diabetes had significantly higher age, BMI, blood pressure, sedimentation rate, and serum levels of HbA_{1c}, C-reactive protein, total cholesterol, low-density lipoprotein cholesterol, and triglycerides (Table 1). The difference in C-peptide level between the type 1 and type 2 diabetic groups is according to the definition. The 5 controls differed significantly from the matched diabetic patients with respect to serum levels of C peptide; C-reactive protein; coagulation factors II, VII, and X; low-density lipoprotein cholesterol; and total cholesterol (Table 2). The BMI differed statistically significantly among the 3 groups, and the BMI in patients with type 1 diabetes was lower compared with that in controls ($P < .001$).

WOUND HEALING

No clinical signs of infection developed around the ePTFE tubes during the experiment.

UNIVARIATE ANALYSIS

Figure 1 demonstrates the deposition of hydroxyproline in granulation tissue as a measure of collagen content in the tubes. The median amount of hydroxyproline was 40% lower in type 1 diabetes (0.70 nmol/mg [0.48-1.04 nmol/mg]) compared with type 2 diabetes (1.16 nmol/mg [0.56-1.63 nmol/mg]) ($P = .03$). However, we found no significant difference between controls (1.35 nmol/mg [0.72-1.88 nmol/mg]) and type 2 diabetic patients for the whole group of 25 patients or for

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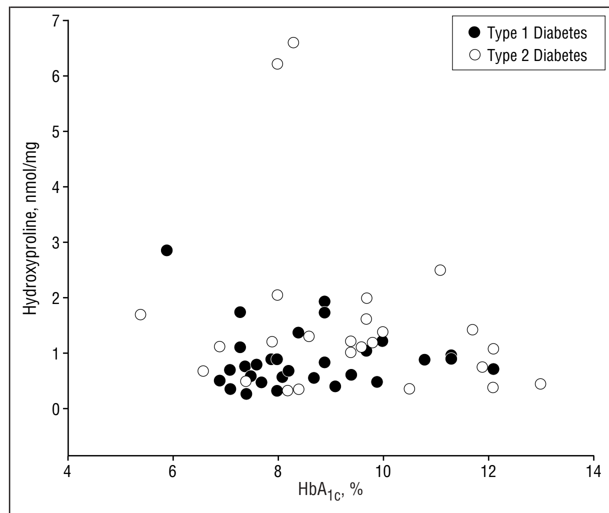

Figure 2. The amount of hydroxyproline deposited in granulation tissue plotted against glycosylated hemoglobin (HbA_{1c}) level. No statistically significant correlation (r_s=0.07; P=.63) was found between hydroxyproline and HbA_{1c} levels.

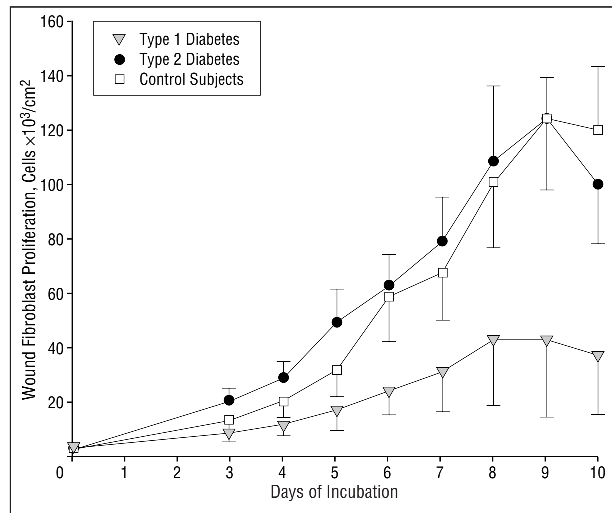

Figure 3. Wound fibroblast proliferation in vitro from patients with type 1 (n=4) and type 2 (n=8) diabetes and control subjects (n=4). Data are expressed as mean±SEM.

the 10 patients matched to controls (1.47 nmol/mg [0.71-3.42 nmol/mg]) (P=.25) (Figure 1). The hydroxyproline deposition in the 10 patients with type 1 diabetes matched with the whole group was 0.85 nmol/mg (0.55-1.16 nmol/mg). Weak but statistically significant positive correlations were found between hydroxyproline deposition and the blood concentration of neutrophils (r_s=0.28; P=.03); coagulation factors II, VII, and X (r_s=0.32; P=.02); and BMI (r_s=0.29; P=.03).

MULTIVARIATE ANALYSIS

Only the type of diabetes (P=.02) and the concentration of neutrophils (P=.03) were independently and statistically significantly associated with hydroxyproline deposition.

IMPACT OF CONTROL OF GLYCEMIA

We found no statistically significant correlation between levels of hydroxyproline and the regulation of glycemia as expressed by HbA_{1c} level in type 1 or in type 2 diabetes (**Figure 2**).

COLLAGENASE

The median collagenase activity in wound-tissue homogenates was 158.8 mU/mg (14.0-242.7 mU/mg) in the type 1 and 81.7 mU/mg (39.8-190.7 mU/mg) in the type 2 diabetic groups, a statistically nonsignificant difference (P=.88).

IN VITRO GROWTH OF WOUND FIBROBLASTS

Fibroblasts cultured from the granulation tissue exhibited similar growth rates in controls and in patients with type 2 diabetes. Type 1 diabetes was associated with pronounced, decreased fibroblast proliferation (**Figure 3**).

COMMENT

The ePTFE tube wound model, originally developed by Goodson and Hunt[26] and refined by Jorgensen et al,[20] has provided valuable data for the healing of wounds in a series of studies. Decreased collagen deposition, which predicts the tensile strength in wound repair,[27] has been reported in uremia,[28] malnutrition,[29] dehydration,[30] and smoking[31] and secondary to treatment with corticosteroids[27] using this model. After major surgery[32] and surgery after preoperative debility and illness,[33] a decreased collagen synthesis has also been reported. For these reasons, the ePTFE tube model appears to be a reliable research tool in the study of wound healing in humans.[34]

The present investigation shows that collagen deposition measured as hydroxyproline is decreased in patients with type 1 diabetes mellitus, whereas it is at a normal level in type 2 diabetes. The comparable collagenase activity in type 1 and type 2 diabetes indicates that the reduced hydroxyproline content reflected a decreased collagen synthesis rather than an increased collagenase activity. The decreased deposition of collagen in type 1 diabetes is consistent with the findings in several studies of decreased collagen synthesis in animals with induced diabetes.[35,36] To the best of our knowledge, only 1 human study has examined collagen deposition in ePTFE tubes during wound repair in diabetic patients.[18] In that study, no difference in collagen formation in ePTFE implants was found between 10 men with well-controlled type 1 diabetes and controls. However, the implants used were low-porosity tubing (Gore-Tex; W. L. Gore & Associates, Inc, Flagstaff, Ariz) that allows less tissue ingrowth than the ePTFE tubes used in our study. Moreover, those tubes were analyzed after 5 or 7 days compared with 10 days in the present study, which limited also the amounts of collagen to be deposited.[20]

The limited number of matched controls in our study implies the possibility that the reference level of hydroxy-

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proline could be lower or higher than that actually found. A lower reference deposition of hydroxyproline would indicate a less decreased level in patients with type 1 diabetes. This is not likely, however, because then patients with type 2 diabetes would deposit hydroxyproline above the reference level. A higher reference level oppositely would indicate that patients with type 2 diabetes also have decreased hydroxyproline levels and that the reduction found in type 1 diabetes is underestimated. For these reasons, we are confident in concluding that hydroxyproline levels and deposition of collagen in patients with type 1 diabetes are decreased.

Hehenberger et al[10] reported similar fibroblast proliferation in healthy human subjects and patients with type 1 diabetes. The fibroblasts in that study were obtained from uninjured skin in the arm, whereas in our study, they were obtained from acute wounds, and fibroblasts from wounds are known to be phenotypically different from those in uninjured skin.[37] In our study, the proliferation of wound fibroblasts decreased in patients with type 1 but not type 2 diabetes compared with nondiabetic controls. Fibroblasts are the main source of collagen, and the number of fibroblasts can be taken for a measure of repair.[38] The decreased fibroblast proliferation and the decreased amount of collagen found in type 1 diabetes in our study indicate that type 1 diabetes per se alters fibroblast function and collagen synthesis capacity.

The mechanism for this decrease in wound-healing capacity is not known. It might, however, be related to decreased insulin secretion. First, animals with diabetes induced by cessation of insulin production, like human patients with type 1 diabetes, consistently show decreased deposition of collagen.[5] Second, it has been shown that collagen production by rat-skin fibroblasts increases dose dependently with insulin treatment.[39]

In the present study, the collagen deposition was not correlated to the glycemia expressed by HbA_{1c} level in type 1 or in type 2 diabetes. As emphasized above, animals with induced diabetes resembling type 1 diabetes show deficient collagen synthesis, but in these experiments, the glycemia was extreme, ie, typically 5 times that of healthy animals[35,40] The associated decrease in wound healing capacity was moderate, and it is perhaps not to be expected that minor variations in glycemia will induce a measurable influence on collagen production. In diabetic obese mice resembling human patients with type 2 diabetes, a decreased collagen deposition was found using the ePTFE model.[41] This wound-healing defect was not corrected by insulin therapy or diet, ie, factors controlling the hyperglycemia. Investigations in nondiabetic animals studying the influence of glucose on collagen synthesis and fibroblast proliferation show diverse results as reviewed recently.[39] Thus, the finding of collagen production as independent of metabolic control is not different from results of animal experiments.

This finding of a lack of influence of metabolic control on collagen synthesis should be regarded with caution. Our patients were not severely hypoglycemic or hyperglycemic, ie, they did not require immediate glycemic correction. The deposition of collagen might be compromised at extreme dysregulation, as in animal experiments.

Another reservation is that the HbA_{1c} values were measured immediately before the experiments. That the experimental situation caused the patients to adhere more rigorously to their metabolic regimen cannot be ruled out. This might improve the glycemic control and possibly maintain a better regulation than that reflected by the preexperimental HbA_{1c} values. This in turn would tend to conceal a relation between HbA_{1c} level and collagen formation.

Moreover, the present experiments were designed to elucidate collagen deposition in the healing of acute wounds and the influence of glycemic control in diabetes per se. It cannot be excluded that glycemic control is beneficial for other factors in the complex process of wound healing. The healing of foot ulcers might be complicated by peripheral neuropathy, infection, and/or ischemia, which is different from the experimental situation. Finally, recent studies have substantially documented that a proper metabolic control decreases the risk for complications,[42-44] including a decrease in glycated skin collagen.[45]

Although we take into consideration the substantial evidence of much benefit of a proper regulation of hyperglycemia, the present investigation does not stimulate a concept of chronic foot ulcers to be cured by meticulous metabolic control. The adequate approach[46] consisted of multidisciplinary treatment, which implies offloading of ulcers by means of total-contact casts, ankle-foot orthoses, and special insoles and shoes; surgical decompression, prompt treatment of foot infections, and revascularization when required; and possible maintenance in a standard wound-healing center setup.[47]

CONCLUSIONS

The deposition of collagen is decreased by 40% in type 1 diabetes, influencing probably the potential of wound healing. In type 2 diabetes, the deposition of collagen was normal. Moreover, we found no influence of glycemic control on collagen deposition in type 1 or in type 2 diabetes. Our results relate to acute lesions in the subcutaneous tissue not compromised by peripheral neuropathy, infection, and/or ischemia.

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Corresponding author and reprints: Per E. Holstein, MD, DMSc, Copenhagen Wound Healing Center, Bispebjerg Hospital, University of Copenhagen, DK 2400 Copenhagen NV, Denmark (e-mail: ph03@bbh.hosp.dk).

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