Skin Collagen Metabolism in the Streptozotocin-induced Diabetic Rat

Enhanced Catabolism of Collagen Formed Both Before and During the Diabetic State

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SUMMARY

Collagen catabolism has been measured in skins of streptozotocin-induced diabetic rats. For measuring catabolism of collagen synthesized de novo during the diabetic state, we measured the amounts of [^{3}H]hydroxyproline-containing degradation products in skins of diabetic rats, killed 4 h after [^{3}H]proline injection (protocol 1); degradation products were isolated in TCA-soluble fractions of skin homogenates. For measuring catabolism of collagen preexisting before the induction of the diabetic state, we measured the 21-day loss of [^{3}H]hydroxyproline (and hydroxyproline) in entire skins of rats that were streptozotocin-treated after [^{3}H]proline injection (protocol 2).

A 2.5-fold increase in the relative amounts of [^{3}H]hydroxyproline-containing degradation products was measured in the TCA-soluble fractions of skins from diabetic rats (protocol 1). These degradation products had a low molecular weight (as evident from their diffusibility), and they were derived from recently synthesized collagen, possibly procollagen (as evident from their high [^{3}H]hydroxyproline specific activity). Furthermore, they were not derived from the degradation of [^{3}H]hydroxyproline-labeled collagen present before induction of the diabetic state (protocol 2). Evidence for this conclusion is as follows: the amounts of [^{3}H]hydroxyproline-containing degradation products in skins of diabetic rats were not greater than that in skins of control rats, despite a 50% resorption of collagen in skins of diabetic rats.

Overall, the catabolism of collagen formed de novo during the diabetic state was distinguished from the catabolism of collagen formed before, and both catabolic processes were enhanced in rat skins of streptozotocin-induced diabetic rats. DIABETES *31*:426–431, May 1982.

Streptozotocin-induced diabetes elicits a tissue-specific effect on collagen metabolism. A net loss of collagen mass occurs in skin,[1] whereas a net accumulation of collagen mass occurs in intestine[1] and most likely in glomerular basement membrane.[2] In more exact terms, an enhanced collagen catabolism contributes to the net loss of skin collagen,[3] whereas an enhanced collagen synthesis probably contributes to the net increase of collagen in small intestine[3] and glomerular basement membrane.[4–6]

Diabetes could affect collagen catabolism in at least three stages during its life cycle: (1) on intracellular procollagen; (2) on recently synthesized extracellular collagen before its maturation into fibers; and (3) on mature collagen fibers. The catabolism of procollagen has been quantitated by measuring the amounts of hydroxyproline-containing degradation products;[7] the degradation of recently synthesized extracellular collagen and of mature collagen has been measured by the decay kinetics of prelabeled collagen, especially when the reutilization of labeled proline (or recycling) has been minimized.[8]

When assessing the catabolism of procollagen, by measuring its degradation products, the low-molecular-weight hydroxyproline-containing material must be shown not to arise artifactually during tissue processing. We have taken precautions in this present study to minimize such artifactual proteolysis. Thus, skins of [^{3}H]proline-injected rats were homogenized in the presence of Pepstatin A, and trichloroacetic acid (TCA) was immediately added to the homogenate. With this approach, we have assayed collagen degradation products in TCA-soluble fractions of skins from control and diabetic rats using a protocol that labeled collagen synthesized during the diabetic state (protocol 1). The amounts, specific activity, and molecular size of [^{3}H]hydroxyproline-labeled material in the TCA-soluble fractions of skins from diabetic rats indicate that intracellular catabolism of procollagen was enhanced. Furthermore, collagen synthesized before the rats became diabetic was eliminated as being the source of this material, because only small amounts of low-molecular-weight, [^{3}H]hydroxyproline-con-

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taining material were present in rapidly resorbing skins of diabetic rats that had been prelabeled with [^{3}H]proline (protocol 2). A preliminary report of the results described in this article has been presented.[9]

MATERIALS AND METHODS

ANIMAL TREATMENTS

Protocol 1. Eight male, Sprague-Dawley rats [4 mo old; 406 ± 13 g (± SD)], individually caged, were fed ad libitum for the duration of the experiment (except for 12 h of fasting preceding the time of streptozotocin treatment). On the day of streptozotocin treatment (day 0), four of the rats were lightly anesthetized (ether-inhalation) and injected (80 mg/kg body wt) with streptozotocin (Upjohn Chemical Co., Kalamazoo, Michigan) via the tail vein. Just before injection, streptozotocin was dissolved in isotonic sterile saline containing 1% citric acid, pH 4.5. Four uninjected rats served as age-matched untreated controls; they were neither anesthetized nor injected with isotonic saline, but they were fasted as were the diabetic rats.

Twenty days after streptozotocin treatment, diabetic and control rats were injected (i.p.) with 1500 μCi of L-[2,3-^{3}H]proline (26.6 Ci/mmol; NET 323, New England Nuclear, Boston, Massachusetts) dissolved in isotonic saline. Four hours later, all rats were lightly anesthetized, and blood samples were taken from each by cardiac puncture; all rats were then killed (excess ether-inhalation), frozen in liquid nitrogen, and stored at −35°C. Serum and urinary glucose were determined as described previously.[1] Urinary glucose[1] (data not shown) was greater than +2% for streptozotocin-injected rats from day 1 to day 20, the last day of the experiment.

Protocol 2. Each of eight rats (from the same shipment used in protocol 1) were injected (i.p.) with 1000 μCi of the [^{3}H]proline. Aliquots (0.1 ml) of the isotope were injected on three successive days (−3, −2, and −1) into lightly anesthetized rats with one injection in the morning and one in the afternoon; serial injections were used to achieve greater labeling of tissue collagen than was expected from a single injection. On day 0, four rats were treated with streptozotocin, as described above; 21 days later, all rats were killed and processed (as described for protocol 1).

Tissue preparation. Entire skins (except over head, tail, paws, and genitals), excised from thawed and shaven rats, were divided longitudinally along both dorsal and ventral midlines to yield two half-sections. One half-section from each rat was trimmed with a blunt scalpel to remove excess fat and stored frozen for subsequent homogenization; the removed fat did not contain hydroxyproline (data not shown). The other half-section was also trimmed of excess fat and then lyophilized for measurement of dry weight. Thus, values for the wet and the dry weights of the entire skins (Table 1) do not include excess fat. Skins from diabetic rats were similarly prepared; however, trimming of excess fat was not necessary, at time periods beyond day 7, because their skins were relatively fat-free.

Homogenization of skin and separation into TCA-soluble and TCA-insoluble fractions. Thawed, half-skin sections (see above) were cut into strips. The strips were minced into pieces (2 × 5 mm), and the pieces were thoroughly mixed to minimize the problem of tissue sampling; 1.5 g of skin pieces was suspended in 30 ml of 0.5 M acetic acid containing Pepstatin A(1 μg/ml, Sigma Chemical Co.) and 30 min at 4°C allowed for the skin to become saturated with the solution. Skin suspensions were then homogenized with a Polytron homogenizer (Brinkman Instruments) fitted with a microtip (no. 6 setting, 30 s, 4°C). After tissue homogenization, the tip was inserted into a test tube containing 5 ml of 0.5 M acetic acid and Pepstatin, and washed by "homogenization" for 5 s.

To the homogenate plus wash, an equal volume of a concentrated (16%) solution (4°C) of TCA (trichloroacetic acid) was immediately added and mixed;[4] after 1 h at 4°C, the suspension was centrifuged (13,200 × *g*, 1 h) to separate it into TCA-soluble (supernatant) and TCA-insoluble (residue) fractions. The residue, mixed with 40 ml of 8% TCA (4°C), was recentrifuged, and its supernantant fraction was combined with the first TCA-soluble fraction. The TCA-soluble and TCA-insoluble fractions were freed of TCA by extracting with excess ether (at least 2 vol used for the TCA-soluble fractions). Both fractions were then lyophilized. The TCA-soluble fraction dried to a brown semi-solid, whereas the TCA-insoluble fraction dried to a white powder. To determine the approximate molecular size of [^{3}H]hydroxyproline-containing material in the TCA-soluble fractions, we

TABLE 1
Comparison of various parameters for rats used during both experimental protocols

Parameter†	Protocol 1*		Protocol 2*		
	Day 20 control	Day 20 diabetic	Day 0 control	Day 21 control	Day 21 diabetic
Body weight (g)	417 ± 16	293 ± 15	404 ± 11	452 ± 30	290 ± 13
Serum glucose (mg/dl)	104 ± 7	343 ± 22	100 ± 12	102 ± 33	402 ± 21
Skin weight (g)					
Wet	68 ± 3	32 ± 2	64 ± 5	74 ± 9	35 ± 10
Dry	ND‡	ND‡	36 ± 7	33 ± 6	12 ± 5
Hydroxyproline (mg/entire skin)	1293 ± 233	703 ± 23	1415 ± 317§	1290 ± 260	772 ± 116
Collagen concentration (% dry weight)	ND‡	ND‡	30 ± 1	29 ± 5	56 ± 7
[^{3}H]Hydroxyproline (dpm × 10^6/entire skin)	13.1 ± 2.5	2.2 ± 0.3	18.6 ± 1.6	16.6 ± 5.4	12.0 ± 0.5

* For a description of protocols 1 and 2, see METHODS.
† All results are expressed as the mean (± SD, N = 4).
‡ Not determined.
§ Previously, we reported[9] a lower value for skin hydroxyproline at day 0. However, this lower value resulted from overscraping in an effort to remove fat. In the present studies, skins were simply trimmed of excess fat (see METHODS), lyophilized, and 1-g quantities were hydrolyzed for analysis of hydroxyproline and [^{3}H]hydroxyproline.

dialyzed (4°C) half the volumes of each TCA-soluble fraction (before they were lyophilized) against several changes of 0.1 M acetic acid.

The TCA-method for isolating low-molecular-weight [^{3}H]hydroxyproline-containing material, in addition to being used to minimize proteolysis during tissue processing (see RESULTS), appears superior to a previously used method involving dialysis of the entire homogenate.[3] The TCA-method is faster and is not affected by substances (present in a concentrated diffusate) that interfere with the colorimetric hydroxyproline assay. The apparent lack of interfering material in the TCA-soluble fraction allows the hydroxyproline assay to be linear over a wider range than possible when assaying a dried diffusate.

The dried TCA-soluble fraction was hydrolyzed in 5 ml of 6 N HCl (as previously described),[1] dried, and dissolved in 2.5 ml of 2 N HCl. A portion (0.5 ml) of the 2 N HCl solution was dried, dissolved in 1 ml H_2O, and diluted for the assay of hydroxyproline. The remaining 2 ml of the 2 N HCl solution was applied to an ion-exchange column (see below) for the determination of [^{3}H]hydroxyproline specific activity. The TCA-insoluble fraction was similarly processed for the analysis of hydroxyproline and [^{3}H]hydroxyproline except for the following modifications: (1) the entire TCA-insoluble fraction was hydrolyzed in 50 ml of 6 N HCl; (2) after hydrolysis, the entire hydrolysate was diluted to 2 N HCl; (3) samples to be assayed for hydroxyproline were diluted at least 20-fold to avoid inhibition.

Analytic methods. [^{3}H]Hydroxyproline was separated from [^{3}H]proline and its specific activity assayed as described previously[1] with a column (0.7 × 23 cm) containing Dowex 50-X12 (200–400 mesh) equilibrated and eluted with 2 N HCl. All samples were counted in a Beckmann LS-100C scintillation spectrometer (Beckman Instruments, Fullerton, California) with an efficiency for tritium of approximately 25%. All values for cpm were converted to dpm by correcting for quenching using the combined external standard-channel ratios method. Total activity of [^{3}H]hydroxyproline in a skin sample was obtained by multiplying the specific activity (dpm/μg) of column-eluted [^{3}H]hydroxyproline by the total amount of hydroxyproline in the sample hydrolysate. This method should obviate problems associated with incomplete column recoveries of [^{3}H]hydroxyproline, such as the conversion of trans to cis-4 hydroxyproline.[9a] The minimum amount of hydroxyproline measured in all samples was 1 μg/ml, and the minimum amount of radioactivity measured was 100 cpm. For these studies, we have assumed that hydroxyproline is present exclusively in collagen; the small amounts of hydroxyproline in elastin and ClQ were considered to be insignificant. The amounts of tissue hydroxyproline were multiplied by 7.46[10] to calculate the equivalent amount of tissue collagen.

RESULTS

CHARACTERISTICS OF THE DIABETIC STATE

Body weight and serum glucose. Diabetic rats, at 20 days (protocol 1) or 21 days (protocol 2) after streptozotocin treatment, weighed approximately 25% less than rats at day 0 (Table 1). In contrast, the weights of control rats remained almost unchanged or increased slightly from day 0 to 20–21 days later. Additional weight losses for diabetic rats were not evident beyond day 21.[3] All streptozotocin-treated rats at day 20 (protocol 1) and day 21 (protocol 2) were hyperglycemic (Table 1) and glucosuric (see METHODS).

Skin weights and hydroxyproline content. The dry weight of skins from streptozotocin-treated rats, at either day 20 or 21, was approximately one-third that of skins at day 0 (Table 1); surprisingly, the further loss of skin weight between days 21 and 56 was just an additional 10% of the day 0 value.[3]

The total hydroxyproline content of skins from diabetic rats at day 20 (protocol 1) and at day 21 (protocol 2) was nearly 50% less than that of control rats at day 0 (Table 1). Thus, the skin of a streptozotocin-induced diabetic rat can be classified as a collagen resorbing tissue. Interestingly, the loss of collagen must have been less rapid than that for the noncollagen constituents of skin, because skins from diabetic rats became enriched in collagen. For example, at day 21 the collagen concentration per dry weight was 56% for skins of diabetic rats (Table 1), whereas in skins of control rats the collagen concentration (29%) was unchanged from that at day 0. At times past day 21, values for collagen concentration in skins of diabetic rats became closer to those of control rats, possibly because of a continued collagen resorption.[3]

Skin [^{3}H]hydroxyproline content. The injection of [^{3}H]proline 20 days after rats were treated with streptozotocin (protocol 1) resulted in 83% less [^{3}H]hydroxyproline in skins of diabetic rats than that in skins of control rats (Table 1). This marked difference, reported earlier,[1] could be due[11] to any or all of the following differences between control and diabetic rats: (1) a reduced rate of skin collagen synthesis, (2) an expanded proline pool size in diabetic rats diluting the specific activity of injected [^{3}H]proline,[4] (3) the loss of injected [^{3}H]proline because of polyuria,[12] and (4) increased degradation of recently synthesized collagen (see below). At present, the quantitative contribution from any of these causes, to the reduced amount of [^{3}H]hydroxyproline in skins of diabetic rats, has not been evaluated and is not critical for understanding the results of this present study. Thus, the distributions expressed as a percent of [^{3}H]hydroxyproline between TCA-soluble and TCA-insoluble fractions of skins from diabetic and control rats were comparable to each other, because the distribution should be independent of the absolute amounts of [^{3}H]hydroxyproline in the tissue.

In contrast, the amounts of [^{3}H]hydroxyproline remaining in skins of diabetic rats at day 21 (protocol 2) should reflect collagen degradation because the skins of all rats contained the same amount (18.6 ± 1.6 × 10^6 dpm) of [^{3}H]hydroxyproline at day 0 (Table 1). The skins of diabetic rats lost 36% of [^{3}H]hydroxyproline by day 21 (Table 1). This is less than the 50% loss of unlabeled hydroxyproline (described above) and this difference could be due to the recycling of [^{3}H]proline (see below) that artifactually reduced the loss of prelabeled collagen.

MEASUREMENT OF [^{3}H]HYDROXYPROLINE-CONTAINING DEGRADATION PRODUCTS (AMOUNTS, SPECIFIC ACTIVITY, AND DIFFUSIBILITY)

Protocol 1. The major finding of the present study (Table 2) was that 4 h after [^{3}H]proline injection of diabetic rats (protocol 1) (METHODS), skins of diabetic rats contained a 2.5-fold greater amount of TCA-soluble [^{3}H]hydroxyproline (30.1%) than skins of control rats (13.0%). These relative amounts of

TABLE 2
The amounts, diffusibility, and specific activity of [^{3}H]hydroxyproline-containing material derived from skin collagen that was synthesized *during* the diabetic state (protocol 1)

Marker and distribution	Control (N = 4)	Diabetic (N = 4)
Hydroxyproline (μg*)		
TCA-soluble	131 ± 63 (34 ± 13)†	98 ± 43 (56 ± 14)
TCA-insoluble	28,495 ± 4,893	33,517 ± 1,852
% Soluble‡	0.4 ± 0.2	0.3 ± 0.2
[^{3}H]hydroxyproline (dpm)		
TCA-soluble	35,826 ± 5,941 (78 ± 2)	30,518 ± 1,909 (92 ± 4)
TCA-insoluble	243,774 ± 41,070	71,797 ± 12,071
% Soluble	13.0 ± 2.6	30.1 ± 3.6
Specific activity§		
TCA-soluble	304 ± 101	354 ± 158
TCA-insoluble	9 ± 1	2 ± 0.4

* All results are expressed as the mean (± SD) per 1.5 g wet wt of skin. Hydroxyproline amount refers to that quantity measured after hydrolysis and, thus, includes hydroxyproline that is free and peptide bound.
† Numbers in parentheses are the percent of the TCA-soluble fraction that is diffusible.
‡ μg (or dpm) in TCA-soluble/μg (or dpm) in TCA-insoluble + μg (or dpm) in TCA-soluble × 100.
§ Dpm [^{3}H]hydroxyproline/μg hydroxyproline.

[^{3}H]hydroxyproline were much larger than that for unlabeled hydroxyproline (control, 0.4%; diabetic, 0.3%, Table 2), indicating that the primary source of the degradation products was recently labeled collagen.

The specific activity of [^{3}H]hydroxyproline was approximately 33 times (control) to 177 times (diabetic) greater than the specific activity of [^{3}H]hydroxyproline in their respective TCA-insoluble fractions (Table 2). This further supports the conclusion that a pool of recently synthesized collagen molecules serves as the source of [^{3}H]hydroxyproline in the TCA-soluble fraction.

Most of the [^{3}H]hydroxyproline-containing material in the TCA-soluble fraction (Table 2) was diffusible (78%, control; 92%, diabetic) so that the diffusible material amounted to 10.1% (control) and 27.7% (diabetic) of the total [^{3}H]hydroxyproline in the tissues, similar to the values obtained by the dialysis method [7.9% (control), 26.9% (diabetic)].[3] Thus, measures (Pepstatin added before homogenization and TCA added after) to minimize artifactual proteolysis occurring during tissue processing did not affect the amounts of diffusible [^{3}H]hydroxyproline in skins of control and diabetic rats. Therefore, these degradation products must have existed in situ and were not generated by tissue processing.

Protocol 2. When rats were "prelabeled" with [^{3}H]proline before streptozotocin treatment and killed 21 days later (protocol 2), the amounts of [^{3}H]hydroxyproline in the TCA-soluble fractions (Table 3) were 1.1% (control) and 1.0% (diabetic). Thus, when [^{3}H]hydroxyproline-labeled collagen synthesized before induction of the diabetic state was being degraded (see above and Table 1), the amounts of [^{3}H]hydroxyproline in the TCA-soluble fraction were much less (controls, 1/10; diabetic, 1/30) than that obtained when [^{3}H]proline was injected during the diabetic state (protocol 1, Table 2). Therefore, resorbing collagen synthesized before the diabetic state was but a minor source of [^{3}H]hydroxyproline in the TCA-soluble fraction.

Even with protocol 2 (where the amounts of [^{3}H]hydroxyproline in the TCA-soluble fractions in skins of control and diabetic rats were less than 1%) they still had a greater specific activity than that in the TCA-insoluble fractions. This difference in specific activity could arise from the degradation of a small amount of [^{3}H]hydroxyproline-labeled collagen that was being labeled during the diabetic state from the recycling of [^{3}H]proline; the recycling of [^{3}H]proline from the degradation of noncollagenous proteins is a well-known phenomenon.[8,11] Again, most of the [^{3}H]hydroxyproline in the TCA-soluble fraction of control (57%) and diabetic (75%) rats was diffusible.

TABLE 3
The amounts, diffusibility, and specific activity of [^{3}H]hydroxyproline-containing material derived from skin collagen that was labeled *before* the induction of diabetes (protocol 2)*

Marker and distribution	Control (N = 4)	Diabetic (N = 4)
Hydroxyproline (μg)		
TCA-soluble	159 ± 54 (47 ± 5)	106 ± 23 (75 ± 16)
TCA-insoluble	21,055 ± 1,117	33,273 ± 2,918
% Soluble	0.6 ± 0.2	0.3 ± 0.06
[^{3}H]hydroxyproline (dpm)		
TCA-soluble	2,406 ± 397 (57 ± 22)	3,054 ± 532 (75 ± 28)
TCA-insoluble	216,307 ± 57,330	301,217 ± 35,049
% Soluble	1.1 ± 0.4	1.0 ± 0.3
Specific activity		
TCA-soluble	22.1 ± 7.6	32.3 ± 7.1
TCA-insoluble	10.4 ± 3.2	11.8 ± 4.8

* See legend to Table 2 for details.

DISCUSSION

Use of the degradation-products approach in general. The amounts of low-molecular-weight hydroxyproline-containing material (presumably peptides and free hydroxyproline) in a tissue have frequently been quantitated as an index of collagen degradation, and their presence was concluded to reflect the degradation of recently synthesized collagen more than the degradation of mature collagen.[13–15] Their measurement has involved an analysis of hydroxyproline or radiolabeled hydroxyproline after isolation by dialysis,[3,13,14] ultrafiltration,[15] molecular sieve chromatography,[16] solubility in perchloric acid[17] or, in this present study, by solubility in trichloroacetic acid (TCA). The value of this "degradation product" approach, although limited when applied to an analysis of urine,[11,18] has recently been applied to a wide variety of tissues.

For example, during the incubation of lung explants, labeled hydroxyproline-containing degradation products were produced as soon as 8 min after the addition of labeled proline;[7] furthermore, during cell culture studies, such degradation products were first detected in the cell layer.[19] Both observations strongly indicate that the degradation products are formed close to the time and site of collagen synthesis. More recent studies in vitro[14] and in vivo[3,20] have supported this conclusion. Thus, the maximum amount of labeled degradation products was present closer to the time of labeled proline injection and, thus, closer to the time of collagen biosynthesis. The preceding results are consistent with the origin of the [^{3}H]hydroxyproline-containing material as being recently synthesized collagen, and therefore, likely to be procollagen. However, others[21,22] have suggested their nonpeptide origin. Until this controversy is resolved, we will assume that hydroxyproline only arises from hydroxylation of peptidyl proline and that low-molecular-weight hydroxyproline-containing material only arises from the degradation of such peptidyl material.

The amount of these hydroxyproline-containing degradation products can be conveniently expressed as a percent of the amount of hydroxyproline or [^{3}H]hydroxyproline in the combined TCA-soluble and insoluble fractions. Being thus expressed as a percent, the amounts should be independent of the quantity of tissue analyzed and independent of the total amount of [^{3}H]hydroxyproline in the tissue. This is an important consideration because of the reduced amounts of [^{3}H]hydroxyproline present in skins of diabetic rats, compared with amounts in skins of control rats.

Analysis of collagen degradation products in skins of control and diabetic rats. In our present study, the amounts of [^{3}H]hydroxyproline in the TCA-soluble fraction of skins from diabetic rats, 4 h after [^{3}H]proline injection, were 30% of total tissue [^{3}H]hydroxyproline (Table 2) and 2.5 times more than that observed in skins of control rats (Table 2). Furthermore, most of this material was diffusible. In contrast, although diabetes caused the degradation of at least 36% of prelabeled skin collagen, such degradation contributed little (less than 1%) to the pool of degradation products in the TCA-soluble fraction (Table 3). These results are consistent with the conclusion that low-molecular-weight hydroxyproline-containing material is associated with collagen synthesized de novo rather than the degradation of prelabeled collagen, such as in a resorbing uterine deciduoma.[23]

From these results, we conclude that diabetes increases the degradation of skin collagen synthesized during the diabetic state as well as the degradation of collagen present before induction of the diabetic state (see below). As already stated, we cannot conclude that the exact collagen molecule being degraded is procollagen and that the degradation occurs intracellularly. However, in consideration of the aforementioned studies in vitro, such a conclusion seems tenable, but further experiments will be required to make the conclusion definitive.

Regardless of the site of degradation (intracellular or extracellular), enhanced degradation of collagen synthesized during the diabetic state could explain the greater amounts of older collagen present in skins[24,25] and other tissues[26–28] of diabetic rats, and in human tissues[29] (in which this observation was first made). This enrichment of a tissue with older collagen has been attributed to an increased lysyl oxidase activity,[28,30] although in one tissue where cross-links were analyzed, no such increase in collagen cross-linking was observed.[31]

Comments on the mechanisms for enhanced collagen degradation in skins of diabetic rats. The present results show that streptozotocin-induced diabetes increases the catabolism of collagen formed both before and during the diabetic state.

A general increase of proteolytic activity in skin could cause the enhanced degradation of collagen formed before the induction of diabetes, 50% of unlabeled and 36% of prelabeled collagen by day 21 (Table 3). The increased catabolism of skin collagen, with primarily a structural role, has been observed in both calorie-deficient and in protein-deficient rats.[32] Skin collagen, like muscle proteins,[33] appears expendable in times of stress. A diabetes-induced increase in proteolysis could arise from an increase in lysosomal activity[34] as well as an increase in any of the several enzymes (especially collagenase) involved in collagen degradation.[35] There is evidence for both possibilities.[36,37]

Procollagen has the attributes of a rapidly turning over molecule,[34] being a large glycoprotein of export. Half-lives of 26 min (type I procollagen) and 4 h (type III procollagen) have been reported.[38] Rapid catabolism is also observed when a helix-destabilizing analogue of proline (cis-4-hydroxyproline) is added to fibroblast cultures.[39] In general, rapid catabolism occurs when protein conformation is altered by incorporation of amino acid analogues[40] and as the result of abrupt termination of growing polypeptides by amino acid depletion or by addition of puromycin.[40]

Perturbations that might cause an enhancement of procollagen degradation during diabetes are associated with post-translational modifications such as hydroxylation and glycosylation. Greater hydroxylation[41] and glycosylation[42,43] of collagen has been observed during diabetes. Although under-hydroxylation has been reported not to result in an enhancement of this type of degradation,[20,44] there is one report that an ascorbic acid deficiency does increase intracellular collagen degradation.[45] We are currently investigating the exact nature of the [^{3}H]hydroxyproline-containing degradation products and the reason why they are increased in skins of streptozotocin-induced diabetic rats.

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