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CROSS-LINKED ELASTIN AND COLLAGEN DEGRADATION PRODUCTS IN THE URINE OF PATIENTS WITH SCLERODERMA

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Objective. **To measure the urinary excretion of specific cross-link amino acid markers for mature elastin (desmosine [DES] and isodesmosine [IDES]) and fibrillar collagen (hydroxylysylpyridinoline [HP] and lysylpyridinoline [LP]) in systemic sclerosis (SSc) patients and healthy controls.**

Methods. **Urine specimens from 20 patients with SSc and 22 controls were assessed for DES, IDES, HP, and LP using high performance liquid chromatography and ultraviolet absorption spectroscopy, in combination with an isotope dilution technique in which the urine specimen was spiked with isotopically labeled cross-link amino acids.**

Results. **Mean ± SD levels of urinary DES and IDES were elevated in SSc patients by 2–3-fold, and urinary HP and LP by 3–4-fold, compared with controls (DES 21.0 ± 9.4 versus 7.5 ± 1.4 μg/gm creatinine; HP 109.0 ± 72.9 versus 24.9 ± 5.7 nmoles/mmole creatinine). Nineteen of the 20 SSc patients had urinary DES and HP values that were >3 SD above the control mean. A significant elevation in the HP:LP ratio in SSc patients as compared with controls (mean ± SD 6.9 ± 1.5 versus 5.5 ± 1.3) indicated a soft tissue origin for much of the increased HP.**

Conclusion. **Patients with SSc have higher levels of urinary cross-link amino acids specific for the degradation of mature collagen and elastin. These markers distinguish most SSc patients from healthy controls.**

Systemic sclerosis (SSc; scleroderma) is a disease in which excessive connective tissue is found in the dermis, blood vessels, and internal organs (1). Excessive collagen deposition results from an imbalance between the amount of newly synthesized collagen incorporated into the extracellular matrix by the formation of collagen cross-links and the amount of both newly synthesized and mature collagens degraded. Studies of this imbalance in SSc have focused on excess collagen synthesis deriving from overproduction by fibroblasts. While elastin accumulation has been reported in other fibrotic conditions (2,3), abnormalities of elastin metabolism in SSc have not been reported. Similarly, little work has been done on the in vivo degradation of collagen or elastin in SSc, which might regulate the net amount of deposited protein.

Degradation of mature (cross-linked) elastin can be quantified by measuring the amount of urinary desmosine (DES) and isodesmosine (IDES), cross-link amino acids derived exclusively from mature elastin (4). Degradation of mature fibrillar (types I, II, III, V, and XI) collagen can be quantified by measurement of hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) (5). The cross-link amino acids DES, IDES, HP, and LP are not appreciably metabolized, but are excreted intact in the urine, reflecting mature elastin and collagen solubilization in vivo (6,7).

We measured urinary DES, IDES, HP, and LP in SSc patients and control subjects, with an assay that

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uses an isotope dilution technique (4,8). Significantly elevated levels of DES, IDES, HP, and LP were found in the SSc patients, suggesting enhanced degradation of cross-linked elastin and collagen in SSc.

PATIENTS AND METHODS

Patients and control subjects. Twenty-two age-matched healthy adult subjects, who were lifetime nonsmokers, were recruited from among the employees of Boston University Medical Center, the Boston Veterans Administration Medical Center, Michael Reese Hospital and Medical Center (Chicago), and Case–Western Reserve Medical Center (Cleveland).

Scleroderma patients. Twenty patients with scleroderma according to the American College of Rheumatology (formerly, the American Rheumatism Association) criteria (9) were recruited for this study from Boston University Medical Center, Brown University School of Medicine (Roger Williams Medical Center), Tufts University School of Medicine (New England Medical Center), and University of Massachusetts Medical Center. One patient was a current smoker. The mean ± SD duration of disease since the first symptoms and the time since the onset of skin thickening were 9.8 ± 9.6 years (n = 19; data not available for 1 patient), and 5.0 ± 3.6 years, respectively.

A questionnaire on clinical assessment was completed by each patient's physician. Diffuse SSc was defined as proximal disease with involvement of the trunk, upper arm, or thigh (1). Since cross-link amino acid measurements are normalized for the creatinine content of the urine to correct for variable dilution of urine, we attempted to retrospectively quantify reduced muscle mass in SSc patients. Reduced muscle mass would tend to decrease urinary creatinine excretion and would thus increase cross-link amino acid concentrations normalized for creatinine concentration. Percent ideal body weight was calculated as the ratio of present body weight to ideal body weight × 100. Ideal body weight was determined by the Metropolitan Life Insurance Company Tables (Society of Actuaries, 1959), based on age, height, and a medium frame. Creatinine content was measured using a commercial kit (Sigma, St. Louis, MO).

DES, IDES, HP, and LP measurements in urine. DES, IDES, HP, and LP were measured in urine by our previously described methods (4,8). Aliquots of urine specimens obtained from patients and controls at random times of day were spiked with ^{14}C-DES (500 disintegrations per minute, 1.0×10^4 dpm/nmole) and ^{14}C-HP (300 dpm, 1.1×10^4 dpm/nmole). ^{14}C-DES and ^{14}C-HP were isolated from metabolically labeled neonatal rat aorta smooth muscle cell cultures as previously described (4,10). ^{3}H-LP (300 dpm, 6.4×10^4 dpm/nmole) was also added to selected samples. ^{3}H-LP was isolated from metabolically labeled neonatal rat aorta smooth muscle cell cultures by elastase digestion (10), acid hydrolysis of the resulting insoluble fibrillar collagen, gel filtration of the hydrolysate, and high performance liquid chromatography (HPLC) separation as described below.

The spiked urine samples were subjected to acid hydrolysis, gel filtration, and HPLC. Using a photodiode array detector, eluted material was quantified by ultraviolet absorption spectroscopy at a wavelength corresponding to the absorption maximum for the individual cross-link amino acid. The detector was also used to confirm the purity of the material in the peaks and the shoulders of the peaks by comparing their ultraviolet absorption spectra from 220 nm to 320 nm with those of standard DES, IDES, and HP, which have absorption maxima of 268, 280, and 295 nm, respectively. HP and LP exhibited superimposable ultraviolet absorption spectra; the molar extinction coefficient of 6,920, reported for HP, was employed for both (10). The individual cross-link amino acids were collected and assessed for radioactivity.

The isotope dilution method is based on the principle that the decrease in specific radioactivity of the added ^{14}C-DES is proportional to the amount of endogenous DES present in the sample, i.e., the isotope is diluted by the endogenous DES. The method is not dependent on uniform recovery. Nevertheless, our recovery of ^{14}C-DES from control and patient urine samples was not different (35 ± 8% and 37 ± 12%, mean ± SD recovery from control and patient samples, respectively). Since IDES and DES in the same sample exhibit the same recovery, the recovery of ^{14}C-DES was used to calculate endogenous IDES in the sample (4). Similarly, values for LP were calculated using the same recovery as found for HP, since the fractional recovery of ^{3}H-LP and ^{14}C-HP in urine specimens was found to be equivalent. DES and IDES values were

Table 1. Summary of clinical assessment of the 20 systemic sclerosis (SSc) patients*

Clinical feature	No. affected	No. not affected
Raynaud's phenomenon	19	1
Digital ulcers	12	6
Skin disease (area involved)		
Truncal scleroderma	9	8
Upper arm	9	8
Forearm	11	7
Hand (proximal to MCP joint)	16	2
Fingers	20	0
Face	17	3
Thighs	8	10
Trunk, upper arm, or thigh (diffuse SSc)	12	8
Tendon friction rubs	6	14
Esophageal reflux	17	2
Weight loss >10% in last year	4	9
Cardiac arrythmias requiring therapy	0	17
Shortness of breath	12	8
New hypertension (since onset of SSc)	4	14
Anti-topoisomerase I antibody	8	10
Anticentromere antibody	2	15
Receiving D-penicillamine	6	14

* For several features, numbers do not total 20 because data were unavailable on some patients. Other data collected included serum creatinine (mean ± SD 0.8 ± 0.2 mg/dl, n = 19) and erythrocyte sedimentation rate (mean ± SD 34 ± 25, n = 12). MCP = metacarpophalangeal.

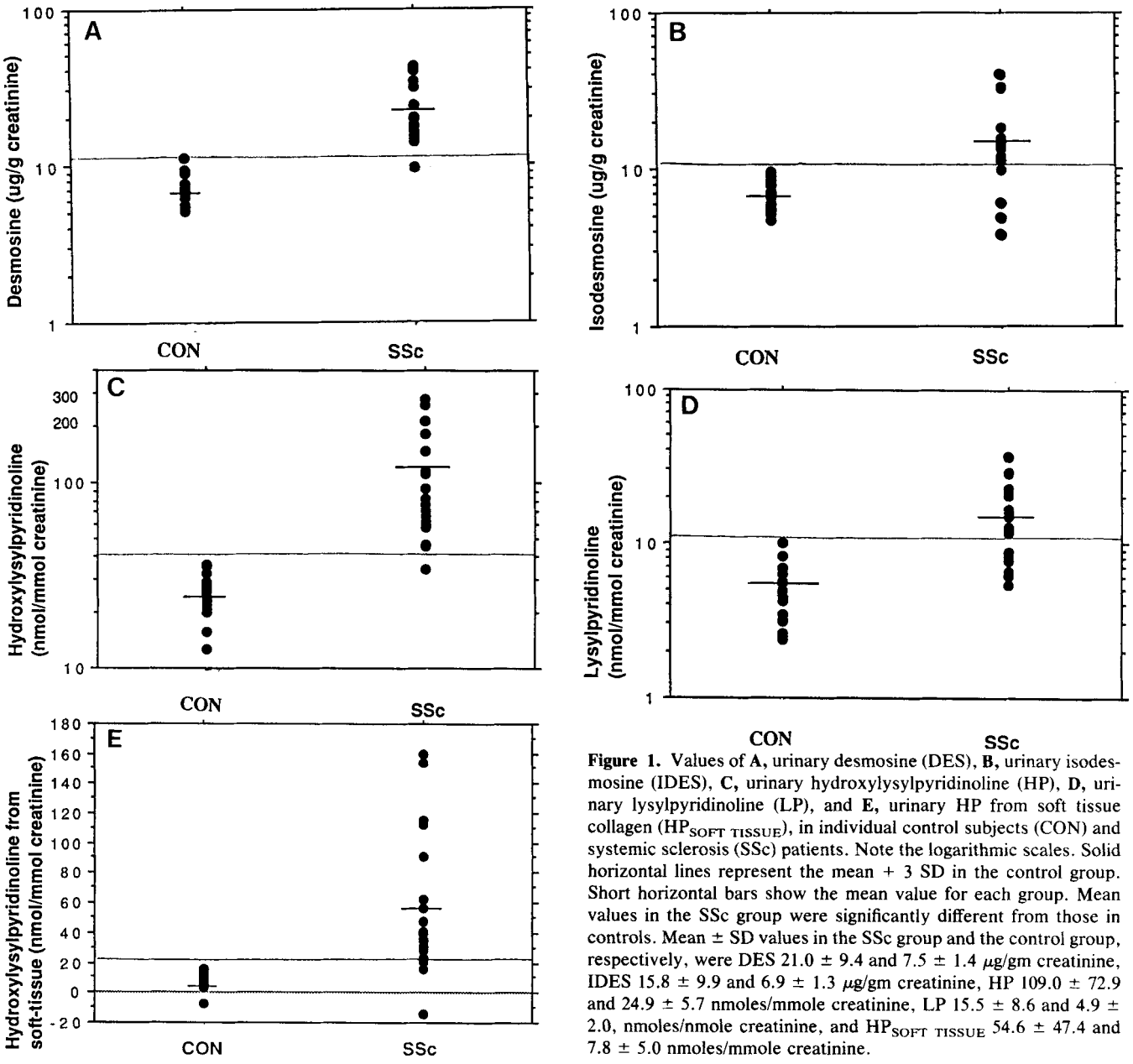

Figure 1. Values of **A**, urinary desmosine (DES), **B**, urinary isodesmosine (IDES), **C**, urinary hydroxylysylpyridinoline (HP), **D**, urinary lysylpyridinoline (LP), and **E**, urinary HP from soft tissue collagen ($HP_{SOFT\ TISSUE}$), in individual control subjects (CON) and systemic sclerosis (SSc) patients. Note the logarithmic scales. Solid horizontal lines represent the mean + 3 SD in the control group. Short horizontal bars show the mean value for each group. Mean values in the SSc group were significantly different from those in controls. Mean ± SD values in the SSc group and the control group, respectively, were DES 21.0 ± 9.4 and 7.5 ± 1.4 μg/gm creatinine, IDES 15.8 ± 9.9 and 6.9 ± 1.3 μg/gm creatinine, HP 109.0 ± 72.9 and 24.9 ± 5.7 nmoles/mmole creatinine, LP 15.5 ± 8.6 and 4.9 ± 2.0, nmoles/nmole creatinine, and $HP_{SOFT\ TISSUE}$ 54.6 ± 47.4 and 7.8 ± 5.0 nmoles/mmole creatinine.

expressed as μg/gm creatinine and HP and LP as nmoles/mmole creatinine, in accordance with the literature. Urinary excretion of DES, IDES, and HP normalized for creatinine excretion does not differ between 24-hour urine specimens and randomly collected urine specimens from the same individuals (4,8). LP has not been investigated, but is assumed to behave as HP. When multiple urine specimens were obtained from a subject, the mean value was utilized in analyses.

Statistical analysis. Data are presented as the mean ± SD. Using Statview 4.01 (Abacus Concepts, Berkeley, CA), comparisons between 2 groups were made by unpaired *t*-test. Comparisons involving 3 or more groups were made using analysis of variance and Scheffe's test. Pearson's correlation coefficients were calculated. *P* values less than 0.05 were considered significant.

RESULTS

The results of the clinical assessment of the SSc patients are summarized in Table 1. Twelve SSc patients had diffuse SSc. With respect to all of the cross-link amino acid parameters, SSc patients differed from controls, both overall (Figure 1) and when

Table 2. Age, sex, body weight, % ideal body weight, and urinary collagen and elastin cross-link amino acid values in the systemic sclerosis (SSc) patients and controls*

	SSc patients		Controls	
	Male (n = 5)	Female (n = 15)	Male (n = 11)	Female (n = 11)
Age, years	53 ± 20	49 ± 12	48 ± 18	38 ± 12
Body weight, pounds	184 ± 44	141 ± 35	177 ± 12	141 ± 6
% ideal body weight	116 ± 27	120 ± 31	116 ± 13	115 ± 5
DES, μg/gm	25.4 ± 11.1†	19.5 ± 8.6†	7.2 ± 1.7	7.8 ± 1.1
IDES, μg/gm	18.4 ± 8.6†	14.7 ± 10.6†	6.0 ± 1.0	7.8 ± 1.1
HP, nmoles/mmole	105.3 ± 87.9†	110.2 ± 70.6†	20.5 ± 4.5	28.5 ± 4.7
LP, nmoles/mmole	14.0 ± 8.7†	16.1 ± 8.8†	3.6 ± 1.1	5.8 ± 2.1
$HP_{SOFT\ TISSUE}$, nmoles/mmole	56.4 ± 58.5†	54.0 ± 45.5†	7.8 ± 3.3	8.1 ± 6.2
HP:LP ratio	7.2 ± 1.6†	6.8 ± 1.6†	5.9 ± 1.3	5.3 ± 1.5

* Values are the mean ± SD. Data were not available on all subjects, as follows: for both body weight and % ideal body weight, n = 14 female SSc patients and n = 10 female controls; for DES, n = 10 female SSc patients; for IDES, n = 13 female SSc patients; for HP, LP, $HP_{SOFT\ TISSUE}$, and HP:LP ratio, n = 8 male controls. Values for urinary DES, IDES, HP, and LP are normalized for the creatinine content of the urine samples. $HP_{SOFT\ TISSUE}$ was calculated from the equation $HP_{SOFT\ TISSUE} = HP_{TOTAL} - (3.5 \times LP)$. See Figure 1 for definitions.
† $P < 0.05$ versus control subjects of the same sex, by Scheffe test.

the comparison was controlled for sex (Table 2). Differences in the cross-link amino acids levels between male and female subjects within a group were not found. With respect to age, body weight, or percent ideal body weight, no differences were found between control and SSc groups, whether or not the comparison was controlled for sex.

Elevation of urinary DES, IDES, HP, and LP levels in SSc patients. Mean levels of DES, IDES, HP, and LP, respectively, in the urine of the SSc patients were 2.8-, 2.4-, 4.4-, and 3.2-fold greater than those in controls ($P < 0.05$ for all), indicating increased degradation of mature elastin and fibrillar collagen (Figure 1). In 19 of the 20 SSc patients, urinary DES and HP levels were >3 SD above the mean for controls. One SSc patient exhibited DES values that were in the control range (4.7–11.2 μg/gm creatinine). The same patient also had HP and LP values in the control range (20.0–36.0 and 3.2–10.2 nmoles/mmole creatinine, respectively, for females). A total of 4 female patients had LP values in the control range. Three patients had IDES values in the control range (4.7–9.7 μg/gm creatinine). Among SSc patients, a correlation was found between urinary HP and DES levels ($r = 0.62$, $P < 0.01$), LP and DES levels ($r = 0.62$, $P < 0.01$), IDES and DES levels ($r = 0.80$, $P < 0.01$), and HP and LP levels ($r = 0.90$, $P < 0.01$) (data not shown).

Urine specimens from 1 of the patients with severe disease were assessed on 2 occasions, 6 months apart. Similar results were obtained for the 2 samples. In the earlier sample and the later sample, respectively, the DES level was 43.9 and 42.4 μg/gm creatinine, the IDES level was 39.9 and 40.1 μg/gm creatinine, the HP level was 319.0 and 244.7 nmoles/mmole creatinine, and the LP level was 38.9 and 34.5 nmoles/mmole creatinine.

Tissue origins of urinary HP and LP in SSc patients. The urinary concentration of LP and HP in 20 SSc patients helped identify possible tissue sources of the excess pyridinoline cross-links. The tissue of origin of LP is almost exclusively bone, and the ratio of HP to LP in bone collagen is 3.5:1 (7). Therefore, the amount of urinary HP that is derived from soft tissue collagen degradation can be estimated by the equation $HP_{SOFT\ TISSUE} = HP_{TOTAL} - (3.5 \times LP)$. The level of soft tissue–derived urinary HP in SSc patients was significantly elevated compared with that in controls (Table 2 and Figure 1). The higher ratio of HP to LP in urine of SSc patients as compared with controls (6.9 ± 1.5 versus 5.5 ± 1.3) was also consistent with a soft tissue origin for much of the increased urinary HP.

The presence of increased urinary DES and IDES, products of elastin degradation, suggested the lung as one potential source of the degradation products measured. Degradation of lung collagen might also be one source of urinary HP. We therefore compared values seen in SSc patients with those in patients with 2 pulmonary disorders, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) (Figure 2). All 3 disorders were associated with elevated levels of DES, IDES, HP, and LP (data not shown). The increase in DES in SSc patients was

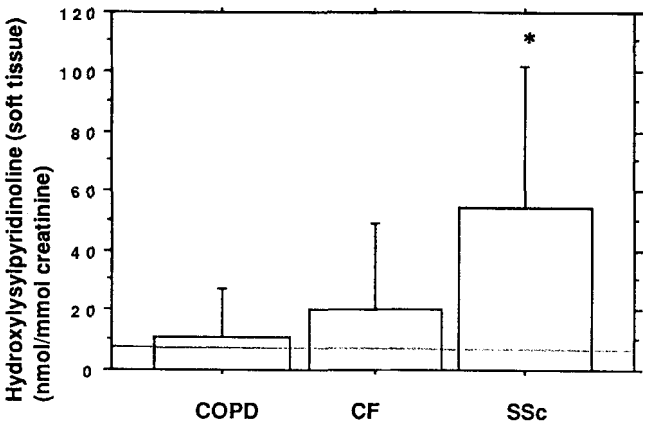

Figure 2. Comparison of levels of urinary $HP_{SOFT\ TISSUE}$ in SSc patients versus patients with chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF). Values are the mean and SD nmoles/mmole creatinine (54.6 ± 47.4 in SSc patients [n = 20], 11.1 ± 16.3 in COPD patients [n = 12], and 20.5 ± 28.8 in CF patients [n = 21]). Solid horizontal line represents the mean value in controls. * = $P < 0.02$ versus COPD and CF patients. See Figure 1 for other definitions.

comparable with that in patients with COPD and CF, but the increase in $HP_{SOFT\ TISSUE}$ in SSc patients was significantly greater (more than 2-fold), suggesting that the lung was not the primary site of $HP_{SOFT\ TISSUE}$. The HP:LP ratio in SSc patients was also significantly elevated as compared with that in COPD and CF patients (6.9 ± 1.5, 4.9 ± 2.1, and 5.0 ± 1.3, respectively). It is unlikely that the increased levels of connective tissue degradation products seen in SSc were due to lung disease from smoking: only 1 of the SSc patients was a current smoker and 9 were former smokers, and there was no difference in their mean DES, HP, LP, or $HP_{SOFT\ TISSUE}$ values as compared with the nonsmokers in the SSc group (data not shown).

Urinary DES, IDES, HP, and LP levels and clinical disease features. An inverse correlation ($r = -0.57$, $P < 0.05$) was found between urinary DES level and single-breath diffusing capacity for carbon monoxide (DLCO) (Figure 3). Patients with diffuse SSc had elevated levels of urinary DES as compared with those without diffuse SSc (24.3 ± 10.8 versus 16.5 ± 4.2 μg/gm creatinine), but the difference was not statistically significant. Similarly, patients with diffuse SSc had urinary HP levels 50% higher than those without diffuse SSc (126.5 ± 78.5 versus 62.8 ± 58.5 nmoles/mmole); however, this difference also did not achieve statistical significance. Skin involvement on the thighs was, however, associated with significantly higher levels of urinary DES (Table 3). Thigh involvement may be a surrogate measure for the total area of skin involvement. Six other areas of skin involvement were investigated, i.e., the trunk, upper arm, forearm, hand, face, and fingers, and the presence of thigh involvement was associated with a mean ± SD of 5.5 ± 0.9 areas affected (n = 8), as compared with 3.3 ± 1.9 for those without thigh involvement (n = 10). The group with tendon friction rubs had significantly higher levels of urinary IDES, HP, and $HP_{SOFT\ TISSUE}$ (Table 3). Weight loss in excess of 10% during the last year was associated with elevation of urinary HP, $HP_{SOFT\ TISSUE}$, and LP (Table 3). Other clinical parameters listed in Table 1 showed no association with urinary DES, IDES, HP, or LP.

DISCUSSION

The mechanism of fibrosis in SSc is thought to result from excess production of connective tissue components (1). We have observed that urinary levels of DES and IDES and of HP and LP, markers for the degradation of mature elastin and fibrillar collagen, respectively, are more than 2-fold higher in SSc patients than in controls (Table 2 and Figure 1). DES, IDES, HP, and LP are excreted in the urine as constituents of the peptides produced by mature elastin and collagen degradation, and their levels should be increased during states of increased degradation. This

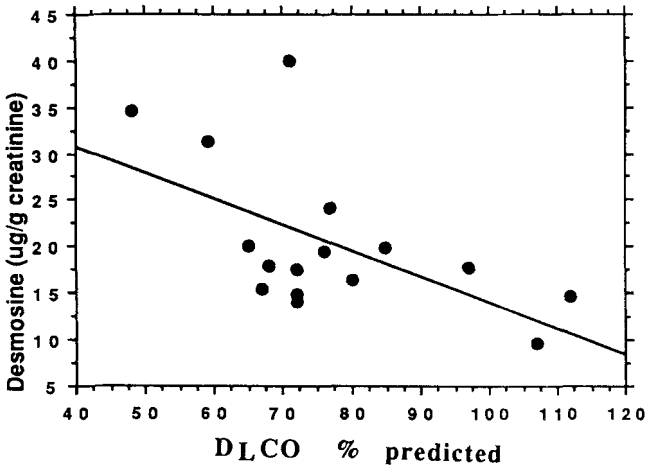

Figure 3. Regression plot of urinary DES levels versus diffusing capacity for carbon monoxide (DLCO) in patients with SSc. The correlation coefficient (r) is −0.57 ($P < 0.05$). The mean ± SD values for DLCO (% predicted) and forced vital capacity (% predicted) were 76 ± 16% (n = 17) and 82 ± 19 (n = 18), respectively. See Figure 1 for other definitions.

Table 3. Association of urinary cross-link amino acid levels with clinical parameters of systemic sclerosis*

Clinical parameter, cross-link amino acid	Parameter present	Parameter absent
Skin involvement on thigh		
DES, μg/gm	28.3 ± 11.9 (7)†	17.1 ± 3.9 (10)
Tendon friction rubs		
IDES, μg/gm	24.6 ± 14.2 (4)†	13.2 ± 7.2 (14)
HP, nmoles/mmole	159.4 ± 75.2 (6)†	87.4 ± 62.5 (14)
$HP_{SOFT\ TISSUE}$, nmoles/mmole	86.5 ± 42.3 (6)†	40.9 ± 43.9 (14)
Weight loss >10% during last year		
HP, nmoles/mmole	210.7 ± 76.7 (4)†	99.5 ± 56.5 (9)
$HP_{SOFT\ TISSUE}$, nmoles/mmole	120.6 ± 47.7 (4)†	49.0 ± 34.5 (9)
LP, nmoles/mmole	25.8 ± 9.0 (4)†	14.4 ± 7.2 (9)

* Values are the mean ± SD urinary cross-link amino concentrations normalized for the creatinine content of the urine samples; values in parentheses are the number of patients. See Figure 1 for definitions.

† $P < 0.05$, versus the "parameter absent" subgroup. Values in both subgroups were significantly greater than those in healthy controls.

is the first report to indicate increased degradation of mature elastin and collagen in SSc. Increased degradation may, of course, result from either an increased rate of degradation of normal levels of collagen and elastin or an increased, normal, or even decreased rate of degradation of pathologically elevated amounts of collagen and elastin. Previous studies, using type III procollagen peptide, investigated markers of collagen that were not specific for mature fibrillar collagen as compared with newly synthesized collagen (11).

With regard to other factors that might have affected these measurements, we and others have found no statistically significant effect of either a meat-free diet or a high-meat meal on the urinary excretion of DES, IDES, or HP normalized for the urine concentration of creatinine (8,12,13). We found that in healthy controls a meat-rich diet caused an increase of 16% and 34% in the creatinine and DES contents, respectively, of the urine (8). When DES, IDES, and HP values were normalized for the urine creatinine content, diet had no effect on the measured amounts. We considered the possibility that the ratios of DES, IDES, HP, and LP to creatinine in SSc patients might have been elevated solely due to reduced muscle mass. Although they are relatively insensitive measures of reduced muscle mass, sex-specific body weight and percent ideal body weight were not different between SSc patients and controls. Serum creatinine levels in the SSc patients were within the normal range (Table 1). The HP:LP ratio, which is independent of urinary creatinine excretion due to a cancelling out of the urinary creatinine content, was significantly elevated in SSc patients as compared with the control group, suggesting a specific effect of SSc, rather than decreased creatinine excretion.

The inverse relationship between elevated levels of urinary DES and DLco % predicted (Figure 3) suggests that the lung may have been an important site of elastin degradation in SSc. A decrease in DLco most commonly reflects the presence of interstitial fibrosis, but may also reflect the presence of pulmonary vascular disease. Other tissue sites of possible elastin degradation include the systemic vascular bed, since involvement of blood vessels is known to occur in SSc (1). Approximately 2% of the skin is elastin (14), and this may also contribute to the elevation of urinary DES and IDES in SSc, as seen by the relationship between skin involvement on the thigh and elevation of urinary DES levels (Table 3). The finding that diffuse SSc was not also associated with elevated levels of urinary DES may be the result of a type I error.

The reason for the relationship between tendon friction rubs and elevation of urinary IDES is not clear. Tendon friction rubs may reflect widespread disease and correlate with both disease morbidity and mortality (Steen V. personal communication).

The relationship between tendon friction rubs and elevated levels of urinary HP suggests that tendon collagen degradation may be one source of elevated urinary HP. For patients who experienced weight loss of >10% during the last year, urinary HP, $HP_{SOFT\ TISSUE}$, and LP were significantly elevated as compared with patients who did not experience this. The elevated urinary levels of these cross-link amino acids (which are normalized for creatinine) may have been exhibited in the former group due to excessive loss of muscle mass. However, both groups had similar levels of serum creatinine (0.8 ± 0.3 mg/dl [n = 4] versus 0.8 ± 0.2 mg/dl [n = 9], respectively) and similar percent ideal body weight (116 ± 34% versus 121 ± 39%, respectively).

We have taken advantage of the tissue specificity of LP to attempt to identify the tissue of origin of the excess collagen degradation observed in patients with SSc. The source of LP, a cross-link amino acid present only in collagen, is almost exclusively bone, where the ratio of LP to HP is 1:3.5 (7). Although LP is also found in human tendon and articular cartilage, with LP:HP ratios of 1:17 and 1:50, respectively, the rate of turnover of collagen in these tissues is usually

low and likely contributes little to urinary LP excretion (7). LP has not been detected in other soft tissues, including lung (7,15), although we have isolated small quantities from fibrillar collagen produced in neonatal rat aorta smooth muscle cell cultures (Stone P et al: unpublished data). Therefore, the elevation of urinary LP is likely associated with the increased resorption of bone in the digits and other areas observed in SSc (16).

The 1:3.5 LP:HP ratio in bone can be used to estimate the amount of urinary HP that derives from soft tissue. The soft tissue collagen degradation in SSc represents a value nearly 7 times that found in controls (Table 2). Approximately 50% of the urinary HP observed in SSc patients likely reflects soft tissue collagen degradation (54.6 of 109.0 nmoles/mmole creatinine). We estimate that, on average, the excess soft tissue collagen–derived HP in SSc patients' urine is derived from the complete degradation of ~0.4 gm mature fibrillar collagen per day, assuming 0.5 nmoles HP/nmole collagen, daily creatinine excretion of 1.5 gm (13.3 mmoles), and a molecular mass for collagen of 300,000 [(54.6 − 7.8) × (13.3 × 1)/(× 300,000 ng)]. Since HP is found primarily in fibrillar collagen (17), this calculation does not take into account the degradation of other collagen types, such as type IV.

The HP:LP ratio and the $HP_{SOFT\ TISSUE}$ value are significantly higher in SSc than in 2 inflammatory pulmonary diseases, COPD and CF (Figure 2). Levels of DES, HP, and LP are not significantly different among the 3 diseases. This observation supports the concept that the increased HP is derived primarily from soft tissue collagen degradation.

Jimenez and coworkers (18), using cultured dermal fibroblasts derived from patients with SSc, found a slight, but statistically significant, increase in the fraction of newly synthesized collagen degraded intracellularly, when compared with normal cells. Cross-linked elastin and fibrillar collagen are susceptible to relatively few proteolytic enzymes, while newly synthesized elastin and collagen are susceptible to proteolysis by a large number of nonspecific proteases (19,20). The possible sources of the elastin- and collagen-degrading enzymes in SSc include inflammatory cells and interstitial cells.

Increased numbers of mast cells have been associated with conditions characterized by inflammation and fibrosis, including SSc (21,22). Mast cells contain a number of proteolytic enzymes, such as an elastase, that are capable of being released in response to agonists (23). However, a mast cell protease capable of degrading mature fibrillar collagen has not yet been characterized. In the lung, the neutrophil may play a role in SSc. Patients with SSc have exhibited increased retention of neutrophils in their lungs and also an augmented influx into the alveolus (24). The neutrophil is known to contain significant amounts of both elastase and collagenase that are released in an inflammatory milieu (25,26). The possible role of interstitial cell–derived proteinases, such as those from the family of matrix metalloproteinases, is not known. However, Uitto et al found that SSc fibroblasts produce normal levels of collagenase in vitro (27).

In summary, we have found evidence of increased degradation of mature elastin and collagen in 19 of 20 patients with SSc. Most striking was the elevation of the portion of the collagen cross-link amino acid hydroxylysylpyridinoline that is associated with soft tissue collagen. Increased degradation of collagen in SSc has not been previously appreciated. Further studies will determine whether these markers will be useful in assessing disease activity in SSc.

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