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Urinary desmosine excretion is inversely correlated with the extent of emphysema in patients with chronic obstructive pulmonary disease

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Abstract

An enhanced proteolysis of lung interstitium is key event in the pathogenesis of emphysema, a major constituent of chronic obstructive pulmonary disease. To assess whether urinary desmosine and/or hydroxyproline may be used as a marker of lung destruction we studied urinary excretions of these products in 20 patients with chronic obstructive pulmonary disease and in 19 appropriate controls in 24 h urine collection samples. For desmosine measurements, we developed a new indirect competitive enzyme-linked immunosorbent assay. The extent of emphysema was measured in high resolution computed tomography (CT) scans, by considering lung area with CT numbers < -950 Hounsfield units (HU).

Urinary desmosine excretion was significantly higher in patients with chronic obstructive pulmonary disease than in controls ($294 \pm 121\,\mu$g versus $183 \pm 93\,\mu$g, $P = 0.003$), and was unrelated with both age and smoking habits. In patients with no evidence or only mild emphysema, desmosine excretion values were significantly higher ($P = 0.006$) than those of patients with moderate to severe emphysema. In patients with chronic obstructive pulmonary disease, urinary hydroxyproline excretion was positively correlated with urinary desmosine excretion but on the average, it was not different from that of controls.

These data indicate that urinary desmosine is a sensitive biological marker of lung elastin catabolism. The relatively low levels of urinary desmosine observed in patients with severe emphysema may be accounted for a decrease in elastin catabolism due to reduced lung elastin mass. Urinary desmosine may be used to identify subjects at risk of developing emphysema and to assess the efficacy of therapeutic interventions. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Urinary desmosine; Urinary hydroxyproline; Emphysema; COPD; HRCT

1. Introduction

Emphysema is characterised by permanent airspace enlargement distal to the terminal bronchiole that is accompanied by destruction of alveolar walls [1]. Degradation of lung tissue structures may affect both elastic and collagen fibres [2–5]. Measurement

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of biological markers of lung destruction has been proposed as a method to study the progression of emphysema in patients with COPD [6,7]. Elastin breakdown (elastin-derived peptides) and collagen degradation products (hydroxyproline) have been reported as markers of lung destruction in emphysema [8–12]. Elastin-derived peptides are breakdown products of mature or nascent elastin, therefore their serum or plasma levels reflect the rate of elastin degradation and re-synthesis, as well as the rate of peptides clearance from the blood [13].

Reportedly, computed tomography (CT) of the chest is the most accurate imaging technique for diagnosing pulmonary emphysema in vivo [14–16]. Comparison between plasma levels of elastin-derived peptides and in vivo assessment of emphysema by CT scanning has yielded conflicting results [10,12]. Desmosine is a cross-linked imino acid unique to mature elastin that is not absorbed from the diet and is excreted solely in the urine [17–19]. Urinary desmosine seems to reflect lung elastin degradation in vivo more accurately than elastin-derived peptides [13,20,21]. A number of studies have dealt with the measurement of urinary desmosine [13,17–23], but only one study has investigated the relationship between desmosine excretion and extent of emphysema as assessed by CT scanning [22].

In order to validate desmosine and hydroxyproline as markers of lung parenchymal destruction, we measured urinary excretion in normal subjects as compared to patients with COPD. Furthermore, we examined the relationship between these two markers and the extent of emphysema as reflected by CT quantitative analysis. For desmosine measurement, we developed a new specific indirect competitive ELISA method. Serum α_1-antitrypsin (α_1-AT) level and plasma elastase-inhibitory capacity were also measured to assess antiprotease status.

2. Material and methods

2.1. Subjects

We studied 20 patients with COPD (16 male, 4 female) who were either current ($n = 6$) or former ($n = 14$) smokers. By selection criteria, all patients had a FEV1/VC ratio lower than 88% of the predicted value [24]. All patients were hospitalised at the time of study. Fifteen of them had acute exacerbation of their respiratory symptoms and the remaining five were evaluated as potential candidates for lung volume reduction surgery. The two out of the 20 patients studied showed reversibility of airflow obstruction defined as a pre- to post-bronchodilator change in FEV1 greater than 15%. Most patients were on inhaled bronchodilator and corticosteroid therapy, and 11 of the 20 were receiving theophylline i.v. and antibiotic therapy.

A group of 19 healthy subjects (15 males, 4 females) with normal pulmonary function tests served as controls. None of them reported occupational exposure or history of lung disease. Thirteen of the control group were current smokers and six were lifelong non-smokers.

2.2. Protocol

All subjects were evaluated uniformly according to a standardised protocol including clinical history, physical examination, pulmonary function tests and chest radiographs. In patients with COPD, CT scanning was also performed. Urine was collected in 0.05% sodium-azide over a 24 h period from all subjects. Samples drawn from the 24 h urine collection were stored at $-20\,^{\circ}$C for subsequent desmosine and hydroxyproline measurements. Blood samples were taken from all subjects to measure elastase-inhibitory capacity and α_1-AT concentration. An informed written consent was obtained from all subjects. The ethics committee of our Institution approved the study protocol.

2.3. Lung function studies

Spirometry (VC and FVC) was performed by means of a pneumotacograph (Fleisch no. 3) linked to a computer (HP-9825A, Hewlett-Packard, Cupertino, CA) according with standard techniques previously described [25,26]. CO diffusing capacity (DLCO, KCO) was measured by the single breath technique using a Morgan respirometer Mk4 apparatus (P.K. Morgan, Chatham, Kent, UK). Data are expressed as percentage of the predicted values [25,27].

2.4. CT scanning

CT scans were performed on a GE SYTEC 3000 scanner (3 s, 160 mAs, and 120 Kvp) with the patients

breath-holding at full inspiration, using 1 mm collimation, and were reconstructed with a bone algorithm (high resolution CT or HRCT). No contrast medium was infused. The lungs were scanned at three pre-selected anatomic levels [26]: (1) top of the aortic arch, (2) origin of the right lower lobe bronchus, (3) 3 cm above the top of the diaphragm. HRCT quantitative analysis included the measurement of the mean CT number in Hounsfield units (HU) as an index of the mean lung density, and the relative lung area occupied by pixels with attenuation values <-950 HU (RA_{950}) as an index of the extent of emphysema.

The CT number is defined as the linear attenuation coefficient of X-rays in a given volume of material relative to the linear attenuation coefficient in water. CT numbers, when expressed in HU, are scaled from 0 HU (attenuation coefficient in water) to -1000 HU (attenuation coefficient in air). Because the lung is a mixture of gas and tissue with a water-equivalent density, its mean CT number is linearly related to the fraction of air in the lung, i.e. the ratio between the volume of air and the volume of air plus the volume of tissue [26].

For HRCT quantitative analysis, regions of interest incorporating the lung fields were outlined manually with a cursor at the three anatomic lung levels taking care to avoid the major pulmonary vessels at the hilum and the boundaries of the lung adjacent to the chest wall, heart, and mediastinum. The total image cross-sectional area from the six regions of interest, the weighted mean CT number, and RA_{950} were calculated by means of a dedicated software program [26]. To quantify the extent of emphysema on HRCT scans, we chose the threshold value of -950 HU as proposed by Gevenois et al. [16]. These authors compared the relative lung area occupied by pixels with attenuation values lower than eight different thresholds (from -900 to -970 HU) with the relative lung area occupied by macroscopic emphysema measured, by a computer-assisted method, in inflation-fixed horizontal lung slices topographically matched with HRCT scan images. The only threshold for which no statistically significant difference was observed between HRCT and pathologic data was -950 HU [16]. Thresholds lower than -950 HU (e.g. -970 HU) underestimated the extent of emphysema, whereas thresholds

higher than -950 HU (e.g. -900 HU) overestimated emphysema when compared with pathologic data [16].

2.5. Urine sample treatment

Samples taken from 24 h urine collection were subjected to acid hydrolysis in 6N HCl (final concentration) at 110 °C for 24 h and desiccated by heating to 65 °C in an air stream. The residue was dissolved in 0.05% Tween 20 in 0.15 M PBS, pH 7.2 (buffer A) and cleared by centrifugation at $1000 \times g$ for 15 min at 4 °C [28]. Supernatants were adjusted to a final pH of 7.2 and 6 for desmosine and hydroxyproline measurement, respectively.

2.6. Urine sample dilution for desmosine assay

Each urinary hydrolysed sample was diluted to a creatinine concentration below a cut-off value of 20 μg per well, as suggested by Laurent et al. [28]. These authors reported that, in urinary samples tested for desmosine content, a creatinine concentration above 20 μg per well is associated with amount of ions or organic compounds that may interfere in the assay [28].

2.7. Enzyme-linked immunoabsorbent assay for desmosine

Rabbit antiserum to desmosine–hemocyanin conjugate (AbI) (Elastin Product Company Inc., Owensville, MO) was incubated with desmosine standard (0–1 ng per well) (Elastin Product Company Inc., Owensville, MO) or with adequately diluted urinary treated samples for 12 h at room temperature. At the same time, microtiter plates (Sigma, Bio Science) were incubated with 0.5 μg of desmosine–albumin conjugate (Elastin Product Company Inc., Owensville, MO) in 0.05 M sodium carbonate buffer pH 9.6 at 4 °C. After incubation, wells were washed five times with 0.05% Tween 20 in 0.15 M PBS, pH 7.2 (buffer A) and saturated with 0.05% Tween 20 in 0.15 M PBS, 1% BSA pH 7.2 for 1 h at room temperature. Triplicate aliquots of AbI-standard or AbI-sample solutions were then added to the wells for a 2 h incubation at room temperature. Wells were then incubated in succession with anti-rabbit IgG (1:2000) (Sigma, Bio Science)

and peroxidase–antiperoxidase (5 μg in buffer A) (Sigma, Bio Science) for 2 h at room temperature. 2,2′-Azino-bis(3-ethyl-benz-thiazoline-6-sulfonic acid) (ABTS) (Sigma, Bio Science) (0.08 mg in 0.1 M citrate phosphate buffer pH 4 containing 0.003% hydrogen peroxide) was then added to the wells. After incubation for 1 h at room temperature, absorbance was read at 405 nm.

The sensitivity and specificity of our ELISA method was assessed by measuring desmosine content in samples containing known amounts of elastin-derived from bovine *Ligamentum nuchae* (Sigma, Bio Science). Elastin samples were digested with porcine pancreatic elastase type III (Sigma, Bio Science) for 48 h at 37 °C and than hydrolysed in 6 M HCl.

The specificity of our ELISA method was also tested for by measuring desmosine content in different fractions obtained from murine lung homogenates according to the method of Peterkofsky and Diegelman [29]. Briefly, lung tissue was homogenised in 5% trichloroacetic acid (TCA) (1:5, w:v) and centrifuged at $3000 \times g$ for 30 min at 4 °C. The pellet, which contains collagen, elastin and other TCA insoluble proteins (TCA insoluble protein fraction) was digested with collagenase type VII from *Clostridium histolyticum* (highly purified and free of non-specific proteases; Sigma, Bio Science) and centrifuged at $3000 \times g$ for 30 min at 4 °C to obtain a supernatant containing collagen peptides (collagenase sensitive protein fraction) and a pellet (collagenase insensitive protein fraction). The latter fraction was further digested with porcine pancreatic elastase type III (Sigma, Bio Science) in order to obtain soluble lung elastin peptides (elastase sensitive protein fraction). Aliquots of TCA insoluble protein fraction, collagenase sensitive protein fraction, and elastase sensitive protein fraction, were then subjected to acid hydrolysis in 6N HCl, and assayed for desmosine content by the method described above.

2.8. Hydroxyproline assay

Aliquots (0.5 ml) of urinary hydrolysate were used for the determination of hydroxyproline according to the method of Kivirikko et al. [30]; data were expressed as milligrams.

2.9. α_1-Antitrypsin assay

Serum α_1-AT levels were measured by the radial immunodiffusion technique by a commercially available kit (Behring, Marburg, Germany); data are reported as milligrams per milliliter.

2.10. Elastase-inhibitory capacity assay

Plasma elastase-inhibitory capacity was determined by the method of Klumpp and Bieth [31]. Succinyltrialanine-*p*-nitroanilide substrate was used as the elastase substrate; values were expressed as unit of inhibition of elastase per milliliter of plasma normalised for α_1-AT concentration.

2.11. Statistical analysis

Data are reported in the text as means ± S.D. All comparisons between the two groups studied were performed using the unpaired *t*-test; correlation between biological markers and lung function or HRCT data was tested for by simple and multiple regression models (step-wise method).

3. Results

3.1. Characteristics of the study population

The characteristics of the study population are reported in Table 1. Compared with the control subjects, patients with COPD were, on the average, significantly older and had a higher cigarette consumption lifetime. The degree of airflow obstruction in patients with COPD ranged from mild to severe; similarly, DLCO and KCO ranged from normal to greatly reduced (Table 1).

In patients with COPD, the mean CT number averaged -873 ± 27 HU (range -912 to -805 HU). The extent of emphysema, as reflected by the RA_{950}, averaged $21.2 \pm 15.2\%$ (range 0.6–50.4%) of total lung cross-sectional area. Thus, in this population of patients with COPD, the extent of emphysema, as assessed by HRCT quantitative analysis, ranged over a wide spectrum from mild to severe. RA_{950} was inversely correlated with DLCO% ($r = -0.74$,

Table 1
Subject characteristics

	Controls ($n = 19$)	COPD ($n = 20$)	P-value
Age (years)	50 ± 5 (45–67)	66 ± 10 (38–78)	<0.0001
Weight (kg)	74 ± 13 (54–97)	65 ± 18 (37–111)	0.0800
Smoking (pack per year)	12 ± 11 (3–40)	41 ± 24 (5–110)	<0.0001
FEV1 (%)	101 ± 13 (79–116)	39 ± 17 (17–74)	<0.0001
FEV1/VC (%)	105 ± 7 (92–116)	50 ± 15 (25–82)	<0.0001
DLCO (%)	95 ± 14 (83–120)	45 ± 28 (9–109)	<0.0001
KCO (%)	94 ± 15 (81–117)	38 ± 21 (6–64)	<0.0001

Data are expressed as mean ± S.D.

$P = 0.0007$) and KCO% ($r = -0.81$, $P < 0.0001$) predictions. Similarly, the mean CT number did correlate with both DLCO% ($r = 0.69$, $P = 0.002$) and KCO% ($r = 0.66$, $P = 0.004$) predictions, but with a much wider dispersion of data concerning the regression.

3.2. ELISA method for desmosine assay

Intra- and inter-assay variation coefficients for our method ranged from 2 to 6%. The standard curve generated by plotting the amounts of standard desmosine (range 0–1 ng per well) added to the reaction mixture against the corresponding absorbance at 405 nm was curvilinear with a mean correlation coefficient of 0.981 ± 0.022 (Fig. 1).

In samples containing known amounts of elastin from *Ligamentum nuchae*, we were able to detect desmosine content down to 0.08 ng per well. These samples contained, on the average 15.93 ± 0.60 μg of desmosine per milligram of elastin—a value quite similar to that expected on the basis of the constant elastin-to-desmosine molar ratio [32]. These data are comparable to those obtained by chromatographic [33] and other ELISA [28] methods.

We measured desmosine content in protein fractions obtained by subsequent digestion processes (TCA, collagenase, and elastase) from five pools of murine lung homogenates (Fig. 2). Desmosine content in the TCA insoluble protein fraction was similar to that measured in elastase sensitive protein fraction (2.52 ± 0.15 μg per lung versus 2.43 ± 0.19 μg per lung, respectively). No detectable amounts of desmosine were found in the collagenase sensitive protein fraction. The elastin content in TCA insoluble protein

fraction, calculated on the basis of the constant elastin-to-desmosine molar ratio [32], was similar to that measured in lungs from age-matched animals of the same strain by a well-standardised method (168 ± 10 μg per lung versus 164 ± 6 μg per lung) [34].
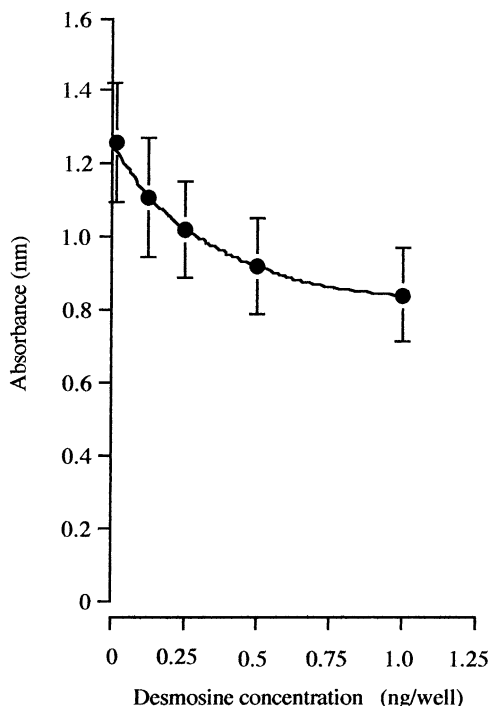

Fig. 1. Standard curve of desmosine ELISA method generated by plotting different amounts of standard desmosine (range 0–1 ng per well) added to the reaction mixture against the corresponding absorbances at 405 nm. Mean correlation coefficient was 0.981 ± 0.022. Data are mean ± S.D. of 12 different experiments.
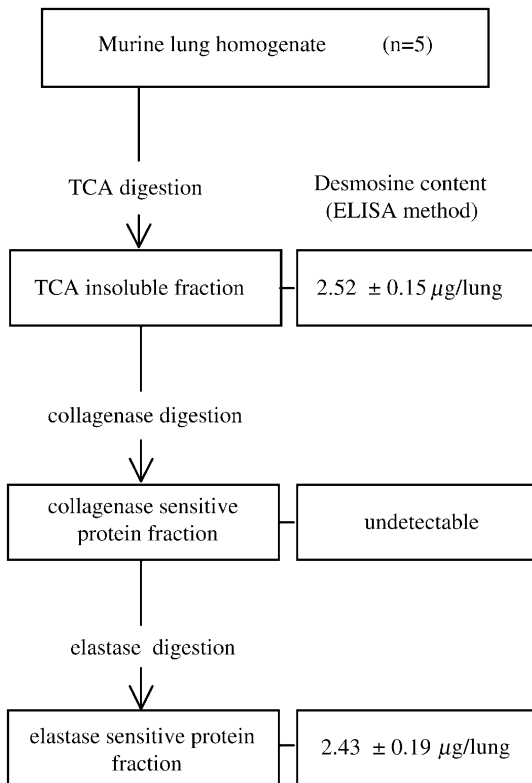
Fig. 2. Specificity of ELISA method for desmosine measurement. Desmosine content was measured in protein fractions obtained by subsequent digestion processes (TCA, collagenase, elastase) from five pools of murine lung homogenates.

3.3. Desmosine and hydroxyproline urinary excretion

Urinary desmosine excretion in control subjects and in patients with COPD is displayed in Fig. 3. In patients with COPD, mean 24 h urinary desmosine excretion was significantly higher ($294 \pm 121 \mu g$) than that of controls ($183 \pm 93 \mu g$, $P = 0.003$). The difference between the two groups was even greater (Fig. 3)

when desmosine excretion values were expressed per gram of urinary creatinine ($333 \pm 112 \mu g/g$ versus $122 \pm 61 \mu g/g$, $P < 0.0001$). It should be considered, however, that urinary creatinine excretion was, on the average, significantly lower in patients with COPD (0.93 ± 0.35 g) than in normal subjects (1.51 ± 0.37 g, $P < 0.0001$). Because normalisation for urinary creatinine introduces a significant bias due to a different muscle mass between the two groups, we used 24 h desmosine excretion for subsequent comparisons.

Table 2 shows urinary desmosine excretion data in relation to the smoking habits. In control subjects, there was no significant difference in desmosine excretion between current smokers and lifelong non-smokers; similarly, there was no difference between patients with COPD who were ex-smokers and those who were current smokers (Table 2). Urinary desmosine excretion values in patients with COPD who had a stable clinical condition did not differ from those of patients who had experienced acute exacerbation of their respiratory symptoms ($289 \pm 118 \mu g$ versus $307 \pm 146 \mu g$, $P = 0.783$). Finally, there was no relationship between urinary desmosine excretion and age in both control subjects ($r = 0.387$, $P = 0.102$) and patients with COPD ($r = 0.049$, $P = 0.836$).

Mean 24 h urinary hydroxyproline excretion in patients with COPD did not differ from that of controls (Table 3). The difference between the two groups became significant when data were expressed per gram of urinary creatinine (Table 3). In patients with COPD, hydroxyproline excretion was directly correlated with desmosine excretion ($r = 0.671$, $P = 0.001$).

In our study, there were no patients with α_1-AT deficiency. Serum α_1-AT and EIC values were mildly but significantly higher in patients with COPD with respect to normal controls (Table 3). However, since EIC normalised for α_1-AT concentration (Table 3) was similar in both groups, we can assume that the same percentage of α_1-AT is functionally active in both groups.

Table 2
Urinary excretion in relation to smoking habits

	Controls ($n = 19$)		COPD ($n = 20$)	
	Non-smokers (6)	Smokers (13)	Ex-smokers (14)	Smokers (6)
24 h desmosine excretion (μg)	147 ± 84 ($P = 0.26$)	200 ± 96 ($P = 0.26$)	275 ± 107 ($P = 0.18$)	368 ± 165 ($P = 0.18$)
24 h desmosine excretion ($\mu g/g$ creatinine)	102 ± 41 ($P = 0.33$)	132 ± 67 ($P = 0.33$)	326 ± 119 ($P = 0.55$)	365 ± 85 ($P = 0.55$)

Data are expressed as mean $\pm$ S.D. Number of patients is reported in brackets.
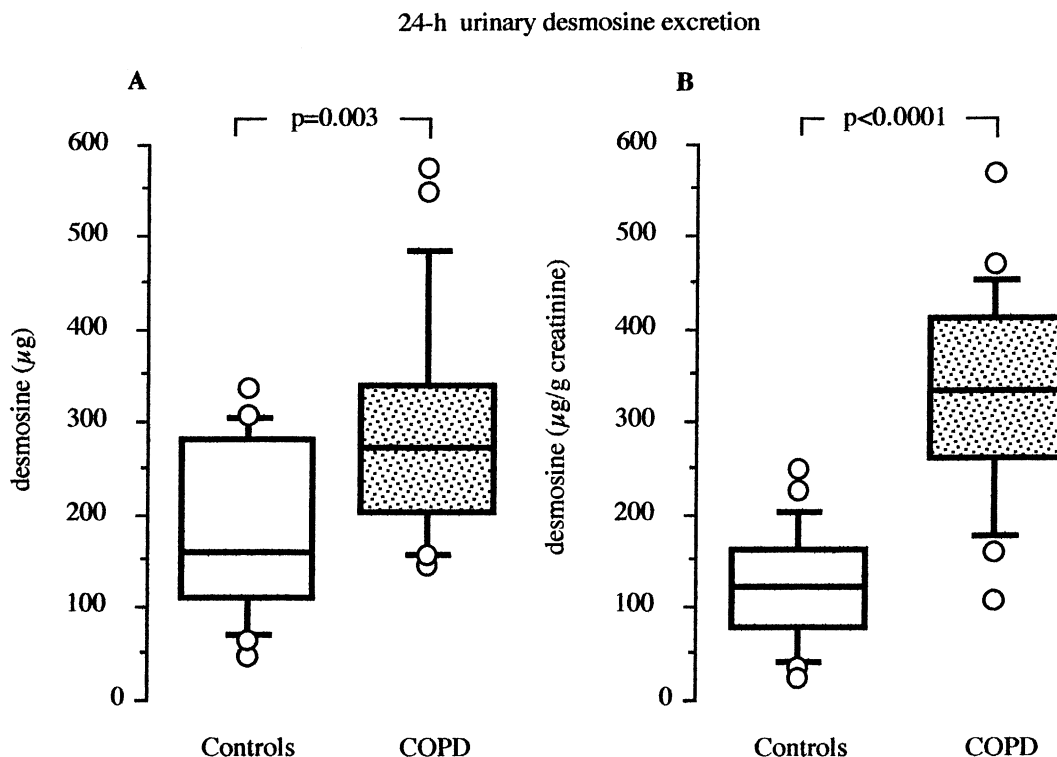
24-h urinary desmosine excretion

Fig. 3. Box-whisker plot of urinary desmosine excretion expressed as 24 h total amount (µg) (A) and per gram of urinary creatinine (B) in 19 subjects with normal pulmonary function (controls) and in 20 patients with COPD. Horizontal line in box: 50th percentile (median); limits of box: 25th and 75th percentile; whiskers: 10th and 90th percentile; circles: outliers.

3.4. Correlation of desmosine and hydroxyproline excretion with lung function and HRCT data

In patients with COPD, 24 h urinary desmosine excretion correlated significantly with FEV1% ($r = 0.52$, $P = 0.019$), FEV1/VC% ($r = 0.57$, $P = 0.009$), DLCO% ($r = 0.64$, $P = 0.005$), and KCO% ($r = 0.64$, $P = 0.006$) predictions. As to the comparison with HRCT data, there was a significant correlation between 24 h urinary desmosine excretion and the mean CT number ($r = 0.66$, $P = 0.002$), the lower the urinary excretion of desmosine the lower the mean CT number, i.e. the lower the mean lung density. This finding is further supported by the

Table 3
Biochemical measurements in the study groups

	Controls ($n = 19$)	COPD ($n = 20$)	P-value
24 h hydroxyproline urinary excretion (mg)	29 ± 9	33 ± 11	0.2789
24 h hydroxyproline urinary excretion (mg/g creatinine)	20 ± 5	38 ± 12	<0.0001
α_1-Antitrypsin (mg/ml)	2.3 ± 0.3	2.8 ± 0.5	0.0007
Elastase-inhibitory capacity (UIE/ml)	8.8 ± 1.5	11.7 ± 2.6	0.0001
Elastase-inhibitory capacity/α_1-antitrypsin (UIE/mg)	4.1 ± 0.9	4.2 ± 0.8	0.5280

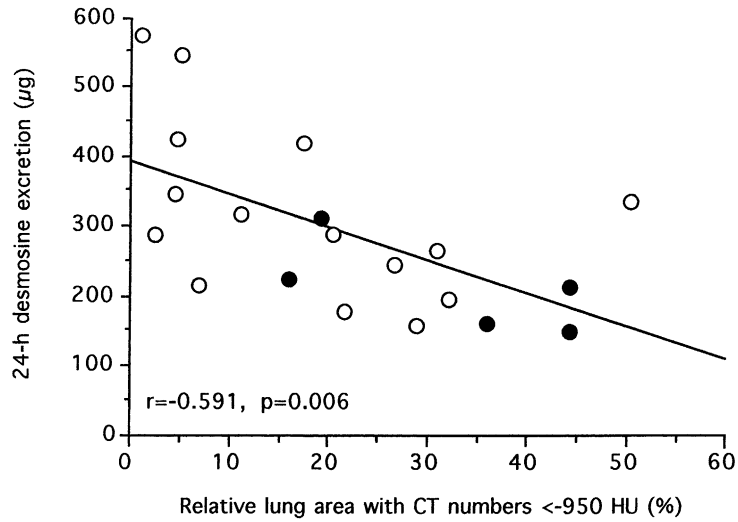Data are expressed as mean ± S.D. UIE: units of inhibited elastase.

Fig. 4. Relationship between percentage of relative lung area with attenuation values <-950 HU and 24 h urinary desmosine excretion in 20 patients with COPD. Solid circles indicate patients who had been evaluated for lung volume reduction surgery.

relationship between 24 h urinary desmosine excretion and RA_{950} taken as an index of the extent of macroscopic emphysema (Fig. 4).

To further analyse this relationship, we divided patients with COPD into two groups as a function of the value of RA_{950}. We used a RA_{950} cut-off value of 15% to differentiate patients with no or only mild emphysema ($RA_{950} = 15\%$) from those with moderate or severe emphysema ($RA_{950} > 15\%$). The cut-off value of 15% was chosen because it represents the 95% upper limit of RA_{950} (mean value $7.8 \pm 4.4\%$) as measured by Gevenois et al. in 42 lifelong non-smoking subjects (mean age 42 years, age range 23–71 years) who had normal pulmonary function tests and no history of lung disease [35]. As indicated in Table 4, there were seven patients with COPD in our study who had $RA_{950} \leq 15\%$ and 13 with $RA_{950} > 15\%$. There was no significant difference between the two groups as to age, pack per year of smoking, or degree of airflow obstruction. However, patients with $RA_{950} \leq 15\%$ had significantly higher 24 h urinary desmosine excretion, DLCO and KCO values than those in whom RA_{950} was greater than 15% (Table 4). Hydroxyproline excretion was positively correlated with the mean CT number ($r = 0.59$, $P = 0.006$), and inversely correlated with RA_{950} ($r = -0.59$, $P = 0.006$). However, these correlations were no longer observed when desmosine excretion was included in the step-wise regression analysis.

Table 4
Lung function data and desmosine excretion in patients with COPD divided in two groups as a function of the extent of emphysema on HRCT

	n	RA_{950} (%)	Mean CT number (HU)	Age (years)	Pack per year	FEV1/VC (% pred)	DLCO (% pred)	KCO (% pred)	24 h DES (μg)
Mild emphysema $RA_{950} \leq 15\%$	7	5.0 ± 3.3	-847 ± 25	65 ± 7	40 ± 32	56 ± 19	68 ± 24	58 ± 6	389 ± 133
Moderate/severe emphysema $RA_{950} > 15\%$	13	29.9 ± 11.2	-888 ± 13	67 ± 11	41 ± 19	47 ± 12	32 ± 21	26 ± 16	243 ± 80
P-value		<0.0001	0.0001	0.624	0.935	0.1705	0.007	0.0004	0.0063

RA_{950}: relative area with attenuation values <-950 HU on HRCT; DES: desmosine; HU: Hounsfield units. Data are given as mean $\pm$ S.D.

4. Discussion

Measurement of biological markers of lung destruction in emphysema (elastin degradation peptides, desmosine, isodesmosine) may be of use to support the diagnosis and to monitor the disease progress. Urinary desmosine excretion is deemed to reflect lung elastin degradation in vivo more accurately than do elastin-derived peptides [11–13,20,21]. We measured desmosine content in 24 h urine samples from normal subjects and from patients with COPD by means of a new indirect competitive ELISA. The ELISA method for desmosine measurement described here is fast and reliable. The sensitivity of the method (0.08 ng per well) allows the use of diluted urine samples so as to minimise the interference due to ions or organic compounds known to cross link in the assay [28]. Our assay is sufficiently specific since, in fractionation studies of murine lung homogenates, comparable amounts of desmosine were detected in the fraction containing soluble lung elastin peptides (elastase sensitive fraction) and in the crude preparation of lung tissue (TCA insoluble protein fraction), which also contained proteins other than elastin (Fig. 2).

We observed that urinary desmosine excretion is significantly higher in patients with COPD with respect to normal controls (Fig. 3). This finding cannot be explained by differences in smoking habits (Table 2) or age. In fact, desmosine excretion was unrelated with these parameters in the two groups studied. Desmosine values measured in normal controls were, on the average, higher than those reported by Stone et al. [13], Stone and co-workers [22], Luisetti et al. [11], and Harel et al. [17]. Such discrepancies may be accounted for by different specificity of the methods. Our method for desmosine measurement probably has lower specificity with respect to the other assays but is much more sensitive than radioimmunoassay [11,23] and more practicable than HPLC or micellar electrokinetic chromatography methods [13,22,23]. The results obtained in our COPD patients are similar to those obtained using other separation methods [36,37].

We found a significant inverse correlation between 24 h urinary desmosine excretion and the extent of emphysema by HRCT (Fig. 4). Namely, patients with no or only mild emphysema had desmosine excretion values significantly higher than those of patients with moderate or severe emphysema (Table 4). These data suggest that, in patients with COPD, an increased elastin catabolism may occur when pulmonary emphysema, as reflected by HRCT, is still mild in extent. On the other hand, the lower urinary desmosine excretion observed in patients with advanced degree of emphysema may reflect a decreased elastin catabolism due to a reduced lung elastin mass [5]. In this connection, it is worth considering that, in the five patients who had been evaluated as potential candidates for lung volume reduction surgery (solid circles in Fig. 4), the 24 h urinary desmosine excretion did not differ from that of normal controls ($210 \pm 65 \, \mu g$ versus $183 \pm 93 \, \mu g$, $P = 0.51$). Recent data reported by Coxson et al. [38] lends support to this hypothesis in as much as they showed that lung tissue mass, as derived from CT lung densitometry, is significantly lower in patients with severe emphysema than in those with mild emphysema and normal controls.

In the literature, there have been only a few studies dealing with the relationship between the rate of elastin and collagen degradation and the extent of emphysema as assessed in vivo by CT scanning [10,12,22]. In two of these studies, the authors measured the plasma or serum concentration of elastin-derived peptides that are not specific markers of mature elastin degradation [10,12]. In a third study, Gottlieb et al. [22] measured elastin and collagen degradation rates in current smokers with and without rapid decline of lung function, using a HPLC assay for urinary desmosine and hydroxyllysylpyridinoline, a specific markers of mature elastin and mature fibrillar collagen degradation, respectively. They found that urinary desmosine excretion was, on the average, 36% greater in rapid decliners than in slow decliners [22]. Among rapid decliners, there was no difference in desmosine excretion between those who had moderate or severe emphysema and those who had no or only trivial emphysema on HRCT [22]. In that study, however, assessment of emphysema was based on a visual score rather than on an operator-independent quantitative analysis. Furthermore, the degree of lung function impairment in rapid decliners with HRCT evidence of emphysema was less severe than that observed in our patients who had a value of $RA_{950} > 15\%$ (Table 4). It is likely, therefore, that the discrepancy between the results reported by Gottlieb et al. and ours may reflect differences in study population as

well as methodological differences in assessing the extent of emphysema on HRCT.

Degradation of lung tissue structure affects both elastin and collagen components. In our study, hydroxyproline excretion was directly correlated with desmosine excretion in patients with COPD. However, hydroxyproline is not suitable as biomarker of emphysema because its relation with the extent of the disease was not confirmed once desmosine was included in the regression analysis. Methodological differences notwithstanding, our findings are in agreement with those of Gottlieb et al. [22] who found no relationship between the rate of collagen degradation and the rate of decline in lung function in current smokers.

In our study, there was no relationship between severity of emphysema and both serum α_1-AT concentration and plasma EIC. Furthermore, there was no difference in the functional activity of α_1-AT between patients with COPD and normal controls. Our data are at variance with those of Fuiita et al. [39] who found that EIC in bronchoalveolar lavage fluid is inversely correlated with the extent of emphysema. It should be considered, however, that EIC in plasma may not adequately reflect the antiprotease activity of the lung tissue, and that it may increase during exacerbations of COPD. In our opinion, this observation deserves further investigation.

In conclusion, our data indicate that urinary desmosine excretion reflects elastin catabolism due to a process of lung parenchymal destruction. The ELISA method described here is capable of detecting differences in urinary desmosine excretion between patients with COPD and normal controls. In addition, we found that patients with COPD and mild emphysema have urinary desmosine values higher than those of both normal controls and patients with moderate or severe emphysema. The technique for measuring desmosine described here may find application in epidemiological studies to identify individuals at risk of developing emphysema, and to assess the efficacy of therapeutic interventions for emphysema.

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