Changes of Biochemical and Biomechanical Properties in Dupuytren Disease

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• ***Background.*—The major biochemical characteristic of Dupuytren disease is the progressive and irreversible deposition of excess fibrous collagen characterized by an enhanced type III collagen proportion.**

***Objective.*—To investigate the influence of changes of the collagen spectrum on the biophysical properties of the palmar aponeurosis.**

***Design.*—Variably affected palmar regions from 30 individuals with Dupuytren disease were classified according to histologic test results and clinical stage. Biochemical, biomechanical, and thermal contracture studies were performed.**

***Results.*—The relative type III collagen content increased with increasing tissue involvement and was found to correlate with calorimetric and biomechanical properties with the exception of the Young modulus. In experiments on the thermal isometric contracture, the collagen denaturation temperature decreased with increasing type III collagen content, ie, increasing involvement. To study the dependence of biophysical properties from the collagen type distribution independent of structural changes, as seen in Dupuytren disease, we investigated rat skins from animals of an age range characterized by dramatic changes in type III collagen content (0–18 months). Biomechanical data also correlated significantly with type III collagen content in rat skin with the exception of the time constant of stress relaxation.**

***Conclusion.*—In light of these results, we suggest that structural changes, such as reduced collagen fibril diameters, associated with alterations in the type III collagen proportion may influence biophysical properties of connective tissues in the involved palmar aponeurosis in addition to alterations of the cross-linking pattern.**

(*Arch Pathol Lab Med.* 2000;124:1275–1281)

Dupuytren disease (DD) is a connective tissue disorder characterized by contracture involving the palmar aponeurosis (PA). The major biochemical features of Dupuytren tissue are an increase of total collagen, increase in relative content of type III collagen and proteoglycans compared with normal palmar fascia,[1–5] lysyl overmodification of type I collagen, an increase of the fibronectin content,[6] and reduced cross-linking of the tissue in comparison with normal aponeurotic tissue.[7] The proportion of type III collagen increases with the degree of involvement of palmar fascia.[1,2,7,8]

The hallmark of Dupuytren contracture is an abnormal proliferation of fibroblasts[9,10] with excessive production of collagen. The extent and type of this proliferation vary according to the stage of the disease. Although in the earlier stages the fibroblasts are the predominant cells within the lesions involving the PA, later stages are characterized by the frequent appearance of myofibroblasts.[11] It has been suggested that these myofibroblasts are involved in the development of the contracture. The traditional view is that in Dupuytren contracture and the early stages of wound healing myofibroblasts are responsible for the abundance of the so-called immature type III collagen.[1] Another reason for the increased type III collagen production was suggested to be the increased fibroblast density found in DD palmar fascia[12] but not a genetic defect in collagen production.[10] The terminal stage of DD, also known as the residual stage, is characterized by mature fibrocytes surrounded by collagen.

As for the biophysical parameters, PA consists of collagen fibers, which have great tensile strength and represent the main load-bearing element, extremely flexible elastic fibers, and proteoglycans. The amount and arrangement of these connective tissue components affect the biomechanical parameters of PA, which have been described in a previous article by our group.[13] These tissue properties are governed by at least 3 distinct parameters: (1) the structure of collagen fibers, ie, orientation and diameter, (2) the nature and density of cross-links, and (3) the interactions of the collagen fiber network with elastic fibers and the proteoglycan matrix. Comparing the biomechanical behavior of normal palmar fascia with that of cords, an increase of residual strain (strain value after complete removal of load), hysteresis loop (area between the loading and unloading branch of the load-strain relationship normalized for strain energy), the time constant, and the viscous stress component was observed.[14] The Young modulus was roughly 2 to 3 times higher in normal tendons (NT) than in PA tissues. Afoke et al[15] reported that the tensile strength for cord tissue was nearly

Accepted for publication February 24, 2000.

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Table 1. Patient Characteristics and Excised Tissues of Different Disease Stages*

Patient No.	Age, y	Sex	Clinical Stage†	2nd Finger	3rd Finger	4th Finger	5th Finger
1	52	M	0, II, III, IV	ANPA	CB	CB	CB
2	61	M	0, II, III	. . .	ANPA	CB	CB
3	47	M	0, I, III	ANPA	TFB	CB	. . .
4	46	M	0, I, IV	ANPA	TFB	CB	. . .
5	58	M	0, I, III	ANPA	TFB	CB	. . .
6	52	M	I, III, IV	. . .	TFB	CB	CB
7	61	M	I, III	. . .	TFB	CB	. . .
8	55	M	0, I, III	. . .	ANPA	TFB	CB
9	53	M	0, III, III	. . .	ANPA	CB	CB
10	52	M	0, I, III	ANPA	TFB	CB	. . .
11	49	M	I, III	. . .	TFB	CB	. . .
12	56	M	0, I, III, IV	ANPA	TFB	CB	CB
13	62	M	0, II, III	ANPA	TFB	CB	. . .
14	61	M	0, 0, I, III	ANPA	ANPA	TFB	CB
15	58	F	0, I, III	. . .	ANPA	TFB	CB
16	57	M	0, I, I, III	ANPA	TFB	TFB	CB
17	62	M	I, III	. . .	TFB	CB	. . .
18	55	M	0, I, III, IV	ANPA	TFB	CB	CB
19	60	M	0, I, III	ANPA	TFB	CB	. . .
20	59	M	0, II	. . .	ANPA	CB	. . .
21	62	M	0, I, III, IV	ANPA	TFB	CB	CB
22	56	M	0, I, III	ANPA	TFB	CB	. . .
23	59	M	0, I, III	ANPA	TFB	CB	. . .
24	53	M	I, III, IV	. . .	TFB	CB	CB
25	60	M	0, II, III, IV	ANPA	CB	CB	CB
26	61	M	0, I, III	ANPA	TFB	CB	. . .
27	58	M	I, III	. . .	TFB	CB	. . .
28	47	F	I, III	. . .	TFB	CB	. . .
29	61	M	I, III, IV	. . .	ANPA	CB	CB
30	59	M	I, III	. . .	TFB	CB	. . .

* ANPA indicates apparently normal palmar aponeurosis; CB, contracture bands; and TFB, thickened fiber bundles.
† Based on Tubiana's classification[21] and evaluated for each finger investigated.

twice that of nodule tissue, but the latter was nearly twice as stretchable as cord. These authors, however, did not study normal control PA. The role of elastin in the modulation of biomechanical properties of DD tissues has already been investigated by us[16]; a significant increase of residual strain and hysteresis loop after digestion of this connective tissue component was demonstrated to exist. Proteoglycans contribute to the viscous stress component in collagenous tissues.[17]

In addition to measurements of biomechanical parameters, thermal isometric contraction is a well-known method for the characterization of the stability of collagen. A tissue sample, the length of which is kept constant, is heated at a constant rate. At a temperature of about 60°C, collagen denaturation is initiated, exerting a significant tension. The extent of the increase in tension and the temperature range in which the phase transition takes place depend on the arrangement of collagen fibers and the density and nature of cross-links. Both parameters, the temperature of the onset of the phase transition and the maximum contraction force, may serve as measures to typify the status of cross-links and collagen structure.[18–20]

The aim of the present investigation was to study the interrelation between chemical alterations of the extracellular matrix (especially changes in the relation between type I and type III collagen) and changes in the biophysical properties of PA from patients with DD as a function of type III collagen proportion. We determined the amounts of type I and type III collagen and correlated them with the thermal contraction force and the viscoelastic behavior of PA.

MATERIALS AND METHODS

Specimens

Normal PA (NPA) and NT were taken from 30 patients with carpal tunnel syndrome. The age of the patients ranged from 46 to 62 years. Specimens of DD were obtained from surgical procedures undergone by patients with DD (Table 1). All tissue samples from patients were obtained with informed consent. Sampling was performed from the variably affected areas of the aponeurotic fascia within the same patients. For histologic studies, specimens were fixed in buffered formalin saline (pH 7.4) for 24 hours, dehydrated, and embedded in paraffin wax. Then, 5-μm sections were cut and stained with hematoxylin-eosin and by the van Gieson technique. Elastic fibers were stained using Sigma elastic stain kit (HT 25-A).

The *histologic classification* of the disease stages was made according to the classifications of Millesi et al[14]: apparently normal PA (ANPA), thickened fiber bundles (TFB), and contracture bands (CB). In the latter group, we further differentiate a stage with active cell proliferation (ACP-CB) and a residual stage (RS-CB), characterized by having only a few cells and ''fused'' TFB. According to these terms, NPA is characterized by well-defined individual fiber bundles and a transparent interstitial connective tissue. At this stage, fiber bundles display a distinct crimp. In the light microscope, ANPA does not appear different from NPA. Elastic fibers were more numerous than in controls. The TFB stage displays the original bundle structure. According to Tubiana's *clinical classification*,[21] contracture is limited to 30° at this stage. Because of the thickening of individual fiber bundles, the epitoneal or peritoneal tissue disappears and the fiber bundles tend to form larger units comparable with tissue alterations caused by nonenzymatic glycation, such as those observed in diabetes mellitus,[22,23] which is associated with DD.[24] The CB exclusively consist of TFB and fused fiber bundles of large diameter. According to our findings, CB showed contractures in the range

of 30° to 150°. In ACP-CB, there are nodules of cellular proliferation that consist mainly of fibroblasts, which originate from perivascular spaces. In some areas, the cells are similar to secretory fibroblasts and continue to produce collagen; other fibroblasts resemble myofibroblasts.[11] In RS-CB, the number of cells is reduced to a few irregularly distributed accumulations. Collagen fiber bundles are thick and disoriented. In general, we chose biopsy specimens of a defined disease stage (eg, ANPA or CB) that consisted of more than 90% of histologic traits characterizing this stage (eg, >90% TFB and fused fiber bundles for CB specimen).

Rat skin samples were obtained from the backs of male Sprague-Dawley rats of varying ages (newborn, 2 months, 6 months, and 18 months).

Collagen Synthesis

Collagen synthesis and total protein synthesis were determined according to Peterkofsky,[25] using collagenase for the digestion of collagen. In this procedure, radioactive peptides obtained from digestion of collagen remain soluble after precipitation of noncollagen protein with 5% trichloroacetic acid–0.25% tannic acid. Data are expressed as disintegrations per minute per microgram of DNA as described by Khalil et al.[26] To calculate percentage of collagen biosynthesis, noncollagen protein disintegrations per minute were multiplied by 5.4 to allow for the much lower protein content of noncollagenous proteins.

Tissue samples were obtained under sterile conditions during surgical intervention. Fat was trimmed from the tissue samples. Tissues were then cut into 5- to 10-mm^3 fragments with scalpels. Tissue culture was performed using 0.1 to 0.3 g of tissue wet weight at a tissue content of 0.1 g/mL. Per 1.0 mL of culture liquid (Dulbecco modified Eagle medium, Gibco BRL, Vienna, Austria), 50 μL of L-[U-^{14}C]proline (9.25 GBq/mmol; 1.85 MBq/mL; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) was added. Tissues were incubated overnight in a CO_2-vented incubator at 37°C. After tissue culture, samples were thoroughly washed (3 times) with Dulbecco modified Eagle medium and tissue sediments dissolved in 1.0 mL of Soluene 100 (Packard, Ill, USA) per 0.1 g of original tissue. After incubation for 3 days at 37°C, the tissues were completely dissolved. A total of 10 to 100 μL of this solution was added to 10 mL of Ready Solve Scintillation Cocktail (Packard), containing 7% acetic acid. Radioactivity was determined using a β-scintillation counter. For quantitation of collagen synthesis, sample aliquots were suspended after tissue culture in 1.0 mol/L Tris-HCl, pH 7.4, containing 5 mmol/L Ca^{++} and 1 mg of collagenase (type VII, Sigma, St Louis, Mo) per 0.1 g of original tissue wet weight. After incubation for 16 hours at 37°C, suspensions were centrifuged at 10 000*g* for 20 minutes, and 10 to 100 μL of supernatants was added to 10 mL of the Scintillation Cocktail. The degree of solubilization of collagen was 85% to 90%. The DNA content of the tissues was determined as described by Burton.[27]

Collagen Type Analysis

Collagen was extracted by limited pepsin digestion. Samples of DD and rat skin were defatted and homogenized under liquid nitrogen using a stainless steel homogenizer. A total of 1 g of tissue dry weight was suspended in 100 mL of 0.5 mol/L acetic acid containing 10 mg of pepsin (from porcine stomach mucosa, twice crystallized, 3500 U/mg of protein, Sigma). Digestion was performed at 4°C with gentle stirring. After centrifugation at 10 000*g* for 1 hour at 4°C, supernatants were stored at −20°C. This digestion procedure was repeated 3 times. Efficiency of collagen digestion was more than 80% as determined by hydroxyproline analysis of the pooled supernatants.[28] Collagen was precipitated from the pooled acidic supernatants by a slow addition of crystalline NaCl to a concentration of 0.9 mol/L. The resulting precipitate was separated by centrifugation and redissolved in 1.0 mol/L NaCl and 0.05 mol/L Tris (pH 7.5). From this solution the collagens were reprecipitated by the addition of crystalline NaCl to a final concentration of 3.5 mol/L. This precipitate was dissolved in 0.5 mol/L acetic acid, dialyzed in the presence of phenylmethylsulfonylfluoride (3 mg/L) to inhibit proteases against several changes of the solvent, and lyophilized. For electrophoretic separation of type I and III collagen, polyacrylamide gel electrophoresis was performed.[29] To this end, 1-mg samples were dissolved in 1 mL of sodium dodecyl sulfate (SDS) sample buffer and heated to 95°C for 2 minutes before sample loading. After SDS gel electrophoresis, the polyacrylamide gels were stained with Coomassie blue, and the relative amounts of type I and III collagens were determined by densitometric scanning, measuring the integrated absorption of each band. Standard runs were performed using pure type I and III collagens.

Biomechanical Analysis

A tensile testing device was designed and built in our laboratory.[16] The apparatus consists of a spindle driven by a gear box motor, a load cell with a maximum load of 20 N and a resolution of 10 mN, a potentiometer to measure the deformation, specimen clamps to which abrasive paper (400 grit) was glued, and a bath containing phosphate-buffered saline. The temperature of the bath was controlled by a microprocessor-based thermostat. For tensile tests the temperature was kept constant at 25°C. The reference length, ie, maximum length of a specimen at zero load, was about 20 mm. We performed uniaxial strain-controlled tests. Strain values were calculated as deformation divided by reference length, ie, relative deformation. Load deformation curves were continuously recorded and converted into load strain curves.

The following test protocol was used. The specimens from the PA were strained at a rate of 5.0% per minute until a level of 2.5% was achieved and then kept constant for the *relaxation test.* During the relaxation phase, the load decreases following an exponential law. The *time constant* τ, which is defined as the inverse of the initial slope, ie, as the tangent of the stress-relaxation graph at the beginning of the test. For viscoelastic materials, the load is composed of an elastic and a viscous component. The *elastic fraction,* ie, final (equilibrium) load divided by initial load, represents a dimension-free parameter. The *viscous fraction* is given as 1 minus the elastic fraction. For the determination of the *maximum Young modulus* in DD samples and controls (carpal tunnel syndrome), the samples were loaded at a rate of 5.0% per minute until a strain level of 10% was achieved. Maximum Young modulus is defined as the tangent in the steepest portion of the load strain curve. This tangent was normalized for the collagen content per unit length, representing a measure for the load-bearing quality of the cross-sectional area of a specimen. The relaxation experiments with rat skins were performed at 20% strain level, which was adjusted at a strain rate of 50% per minute. The skins were strained in the direction of the ''body axis'' of the animals.

Thermal Contraction Experiments

Thermal isometric contraction experiments were conducted at temperatures increasing progressively from room temperature to 90°C at a constant rate of 1°C per minute with the aid of a thermostat. The isometric tension or contraction force developed on heating in the tissue at its original dimensions was recorded and expressed in millivolts.[30] This tension is the consequence of hydrothermal shrinkage of collagen fibers. The temperature corresponding to the onset of hydrothermal shrinkage was termed T_D, and the temperature corresponding to the maximum contraction force was termed T_m. Preliminary assays were performed to demonstrate the reproducibility of the isometric tension determination under varying rates of temperature increase in the saline bath. The temperature increase of 1°C per minute was chosen as the most reproducible heating rate.

Statistical Analysis

The correlation coefficients (r) were computed using the Pearson correlation or Kendall tau. The Student's t test was used for comparison of means from different experimental groups. $P < .05$ was considered statistically significant.

Table 2. Collagen Biosynthesis in Differently Involved Dupuytren Disease Tissues*

Tissues	No.	DNA, mg/g of Dry Weight	Collagen Biosynthesis, %	Collagen Biosynthesis, dpm/μg of DNA × 10^{-2}	Noncollagen Protein Biosynthesis, dpm/μg of DNA × 10^{-3}
NPA	5	1.10 ± 0.12	1.24 ± 0.7	0.40 ± 0.23	0.590 ± 0.123
ANPA	7	1.77 ± 0.43†	1.87 ± 0.9	0.78 ± 0.38†	0.758 ± 0.294
TFB	12	2.33 ± 0.27‡	2.64 ± 0.8	2.15 ± 0.65‡	1.468 ± 0.741‡
ACP-CB	6	5.36 ± 1.98§	3.70 ± 1.0	3.71 ± 1.02‡	1.788 ± 0.651‡
RS-CB	5	1.59 ± 0.41†	2.56 ± 0.8	0.54 ± 0.17	0.380 ± 0.083†

* Results are expressed as mean ± SD. dpm indicates desintegrations per minute; NPA, normal palmar aponeurosis; ANPA, apparently normal palmar aponeurosis; TFB, thickened fiber bundles; ACP-CB, active cell proliferation–contracture bands; and RS-CB, residual stage–contracture bands.
† $P < .05$.
‡ $P < .01$.
§ $P < .001$ vs NPA.

Table 3. Biophysical and Biochemical Properties of Rat Skins From Animals of Different Ages*

Age	T_D, °C	T_m	Viscous Fraction, %	Type III Collagen, %
Newborn	57.0 ± 1.0	61.0 ± 1.0	70.6 ± 2.5	17.3 ± 3.4
2 months	59.5 ± 0.3†	62.0 ± 0.3†	52.4 ± 2.1†	12.5 ± 2.1†
6 months	61.5 ± 0.3†	64.5 ± 0.3†‡	33.3 ± 1.5†	8.9 ± 1.7†
18 months	61.8 ± 0.4†	64.9 ± 0.5†‡	27.9 ± 2.5†	8.7 ± 2.3†

* T_D and T_m denote the temperatures corresponding to the onset of collagen denaturation and maximum contraction force. The viscous fraction is defined as the ratio of the viscous stress component to the overall viscoelastic stress. The type III collagen proportion is given as the percentage of total collagen (type I plus type III). Results are expressed as mean ± standard deviation.
† $P < .05$ vs newborn; n = 4.
‡ $P < .05$ vs 2 months.

RESULTS

Structural Findings

The ANPA branches are characterized by a higher density of elastic fibers than in controls.

Fibroblast cell numbers were higher than in controls. Collagen bundles were oriented mainly in the direction of the aponeurosis. Fibrotic cords in diseased aponeurosis consisted of tense collagen bundles and a large number of fibroblasts, both oriented in the direction of the aponeurotic fascia. In TFB, the average diameter of collagen bundles was smaller than in contracture bands.

Collagen Biosynthesis

Protein and collagen synthesis increased from NPA via ANPA and TFB to ACP-CB, whereas RS-CB showed the lowest noncollagen synthesis, and relative collagen synthesis was similar to that of the TFB stage (Table 2).

Correlation Between Type III Collagen Content and the Biophysical Properties of Rat Skin and Tissues From Patients With DD

Rat skin samples and DD tissues were digested by 4 cycles of pepsin digestion. As shown in Table 3, the type III collagen content decreases with increasing age of the animals. The T_D and T_m, as determined by thermal isometric contraction experiments, increased with age. In general, the changes from 6 to 18 months were much less significant than the changes observed in younger age. The inverse correlation between type III collagen content and T_D was significant at $P < .05$ ($r = -0.675$). The correlation coefficient between type III collagen content and T_m was $r = -0.599$ (nonsignificant). However, the viscous stress component was highly correlated to the type III collagen proportion at a level of $P < .01$ ($r = 0.756$). Figure 1 shows the age dependency of the relative viscous stress component, ie, the viscous fraction, of 3 rat skin samples of different ages.

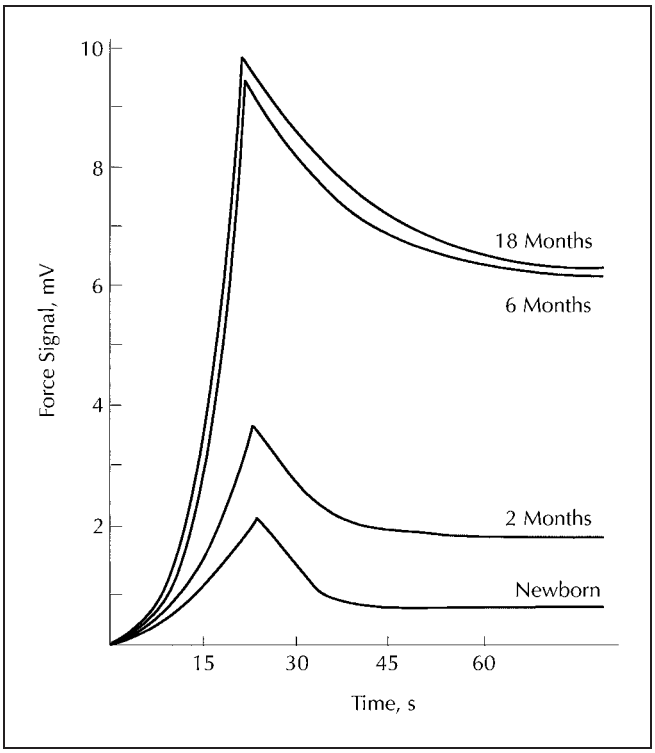

Figure 1. *Stress-relaxation behavior of rat skins from animals of different ages.*

With the exception of the maximum Young modulus, the biomechanical parameters, ie, time constant and viscous fraction measured in DD tissues, show an increase from

Table 4. Biomechanical Properties and Type III Collagen Content of Tendons, Control Palmar Aponeurosis, and Differently Involved Palmar Aponeurosis From Patients With Dupuytren Disease

Tissue	No.	Type III Collagen Proportion, %	Time Constant, s	Viscous Fraction, %	Young Modulus, N·mm/g
NT	7	1.6 ± 0.4	8.6 ± 1.8	1.9 ± 0.3	3100 ± 184
NPA	8	2.8 ± 0.2	12.2 ± 2.1	5.1 ± 1.8	1500 ± 330
ANPA	12	3.7 ± 0.8†	28.1 ± 2.8†	10.6 ± 1.8†	1200 ± 280
TFB	17	15.8 ± 2.9‡	49.1 ± 3.4†	13.4 ± 1.8‡	1100 ± 170†
ACP-CB	11	14.9 ± 2.8	88.0 ± 13.5	15.7 ± 1.9	1350 ± 190
RS-CB	20	19.7 ± 2.1‡	169.0 ± 11.9‡	24.2 ± 1.7‡	1500 ± 210

* Results are expressed as mean ± SD. NT indicates normal tendons; NPA, normal palmar aponeurosis; ANPA, apparently normal palmar aponeurosis; TFB, thickened fiber bundles; ACP-CB, active cell proliferation–contracture bands; and RS-CB, residual stage–contracture bands.
† $P < .05$.
‡ $P < .01$ vs NPA.

Table 5. Relationship Between Clinical Classification and Type III Collagen Proportion*

Dupuytren Disease Stage	No.	Type III Collagen, %
0	9	4.7 ± 0.9
I	11	9.0 ± 1.7
II	12	14.7 ± 2.8
III	20	18.5 ± 4.3
IV	12	22.2 ± 5.1

* Results are expressed as mean ± SD. The type III collagen proportion (percentage of total collagen, ie, type I plus type III) of stages I through IV differ from that of stage 0 at a level of significance of at least $P < .01$. Disease stages were defined according to Tubiana.[21]

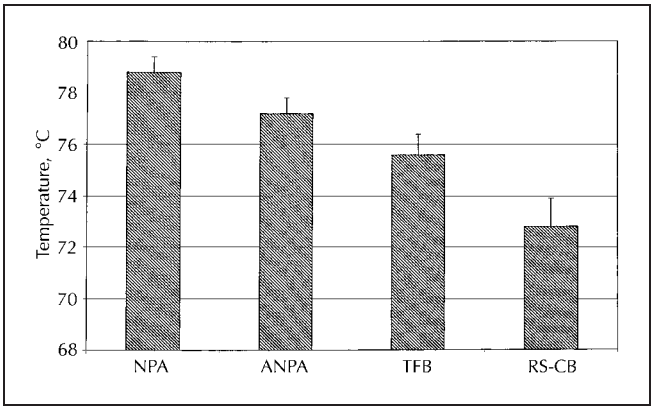

Figure 2. *The temperature corresponding to maximal contracture force as a function of involvement of palmar aponeurosis. All pathologic stages differ significantly from normal palmar aponeurosis (n = 6). NPA indicates normal palmar aponeurosis; ANPA, apparently normal palmar aponeurosis; TFB, thickened fiber bundles; and RS-CB, residual stage–contracture bands.*

NT via NPA, ANPA, and TFB to ACP-CB, which is also reflected in the type III collagen proportion (Table 4). Marked differences between NPA and ANPA and between TFB and ACP-CB were observed with respect to the time constant during stress relaxation. The degree of contracture according to Tubiana[21] correlates with the type III collagen content (Kendall tau, $P < .025$). The contracture stages I to IV show significantly higher type III collagen proportion compared with ANPA (contracture stage 0) with at least $P < .01$ (Table 5). With reference to the thermal isometric contraction experiments, the temperature of phase transition, T_m, decreased with involvement of the tissues (Figure 2). This finding parallels the results obtained with rat skin samples relating to the correlation to type III collagen proportion.

COMMENT

There is general consent that in DD a proliferative phase characterized by clusters of cells actively producing new collagen develops first. The disease then evolves toward a less cellular stage and ends up in a residual fibrotic phase.[31] Increased collagen synthesis is a characteristic feature of DD. In comparison with the histologic stages,[13,14] we observed a clear-cut increase of collagen biosynthesis per unit of DNA from ANPA via TFB to the proliferative phase of CB (ACP-CB). Cell number and DNA content also increased up to ACP-CB. The RS-CB clearly demonstrated the lowest noncollagen protein synthesis per unit of DNA. Interestingly, its relative collagen synthesis attained levels comparable with that of TFB. This indicates that even the ''terminal'' RS-CB stage still comprises some active fibroblasts that have conserved their biosynthetic ''fibrotic'' phenotype. Although total cell content was minimal.

The structure of the individual collagen fibers in the involved regions of DD-PA can be distinguished from that of normal collagen fibers,[32] the major type I collagen fiber bundles being surrounded originally by a sheath of type III fibers. The structure is maintained in apparently uninvolved regions, with the exception that the fascicular sheath is thickened.[33] In TFB, the single TFB fuse to larger units. In CB, the bundle structure is grossly disorganized, and type III fibers are randomly distributed over the cross-sections of the bundles. However, independent of the degree of fibrosis and contracture, the fibrotic cords consist of collagen bundles oriented in the direction of the aponeurotic fascia. In nodules, which because of their focal nature could not be subjected to biomechanical and thermal analysis, there is a loss of fascicular structure and formation of new fine fibrils consisting of type III collagen. In general, DD tissues contain more irregularly arranged fibers with smaller diameters than in normal tissue. According to Notbohm et al,[34] overmodified type I collagen extracted from DD nodules, however, does not produce fibrils of a smaller diameter in vitro. The driving force causing these alterations seems to be the increase in relative proportion of type III collagen. Thus, bundle organization is conditioned by interactions between type I and type III collagen.[35] Experiments by Notbohm et al[36] show that, in agreement with the in vivo observations of an impaired formation of thick fiber bundles in DD nodules, collagen fibrils do not tend to form bundles in vitro when

greater amounts of type III collagen were present in solution.

To clarify the relative importance of type III collagen for modulating the biophysical character of the tissue, we used a model system with rat skins from newborn, 2-month-old, 6-month-old, and 18-month-old rats, which are developmental stages with clear-cut differences in relative type III collagen content but minor changes in collagen structure. In this model we observed an *inverse* correlation between the type III collagen proportion and age or T_m, whereas the viscous stress component was *directly* correlated with the type III collagen proportion. The increase of T_m can be explained by an age-dependent increase in nonreducible thermostable cross-links[37,38] and/or by the decreased type III collagen content of the tissue. According to Allain et al,[39] the changes in the thermal contraction curve in rat skin correlate so well with the nature of cross-links that the role of type III collagen is unlikely to affect the shrinkage characteristics, at least of rat skin, keeping in mind that cross-linking in this collagen is similar to that in type I collagen. Our finding of a dramatically decreasing T_m with increasing type III collagen proportion suggests, however, that for PA tissues from patients with DD type III collagen in addition to cross-linking effects[33] could reduce the thermal stability of the tissue. This could be due to its influence on collagen fibril diameters. As mentioned, DD aponeuroses contain collagen fibrils of significantly smaller diameter in looser aggregation than normal aponeuroses. The increase in the relative proportion of type III collagen possibly plays the major role in the formation of smaller and less organized collagen fibrils, which show decreased T_m values. Of note, the thermal stability of *soluble* type I collagen extracted by limited pepsin digestion from DD palmar tissue was *higher* than that of normal aponeuroses, as shown by circular dichroism measurements.[40] Thermal denaturation of type III collagen was not studied by these authors. It is unknown whether this collagen type has an inherently lower phase transition temperature than type I collagen.

As yet, the potential modifications of biophysical properties by type III collagen and its impact on clinical parameters, such as the degree of contracture, have not been evaluated in DD. We observed similar interrelations between type III collagen content and biophysical parameters as in rat skin (viscous fraction of stress, T_m, T_D), with the exception of the Young modulus and the time constant of stress relaxation (τ), which increased with advancing contracture, ie, in the direction of *increasing* type III collagen content, whereas in rat skin τ increases with increasing age, ie, the direction of *decreasing* type III collagen. This divergence may be explained by the massive structural changes encountered when comparing the different stages of DD from ANPA to CB, changes that are not seen in the developing rat skin. Furthermore, the Young modulus is a clear-cut function of the number and nature of cross-links[41] and less dependent on the collagen type distribution. In DD, the redistribution of type III collagen fibers is closely associated with a relative increase in type III collagen.[33] The concomitant structural changes that lead to contracture might even *result* from the altered collagen type spectrum. Thus, in our hands, type III collagen content in DD tissue correlated closely with clinical stages of contracture (evaluated for each finger involved) as defined by Tubiana.[21] Furthermore, type III collagen proportion increases parallel to increasing involvement of the tissue, as defined by macroscopic clinical appearance supported by histologic inspection.

This work was supported by the Nationalbank Jubiläumsfonds project No. 7584.

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