Changes in the Biochemical Properties of Diseased Tissue as Pathogenetic Factors in Dupuytren's Contracture

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The purpose of this study was to investigate the biomechanical properties of Dupuytren's disease (DD) tissue with special reference to viscoelasticity and to set the result in relation to (a) normal palmar aponeurosis (NPA) and (b) different stages of the disease.

Viscoelastic Properties of Dense Connective Tissue

Ideally, elastic material reacts immediately to any deforming force with a corresponding change of shape and reassumes the original shape as soon as the deforming force stops acting. In reality, however, an elastic material that behaves in this theoretical way does not exist. Especially in living tissues, deformation is always accompanied by a dislocation of internal structures, which occurs against a certain amount of friction and takes some time. Accommodation to a final shape takes place slowly and requires energy. From our preliminary studies, which examined the parameters listed below, we knew that the viscous fraction is low in NPA but significantly increased in tissues obtained from patients with DD. We were not interested in extreme values such as tensile strength, because these values may be influenced strongly by individual factors. We wanted to determine the changes in parameters as expressed in physiological values. We therefore selected strain levels of 2.5%, 5%, 7.5%, and 10%.

Residual Elongation. After the specimens had been elongated to the strain levels mentioned above, they were deloaded, i.e., the strain was reduced. The location where the zero point had again been reached and the length of the residual elongation were then measured. A high residual elongation does not mean that in situ the tissue becomes longer and longer. First, it is quite possible that the residual elongation might spontaneously disappear if one waits long enough. In our study, however, we did not wait. The residual elongation therefore refers to this waiting time. Secondly, before being taken, the specimen is incorporated in the whole tissue complex in situ, where it is exposed not only to longitudinal stress like the isolated specimen after it has been obtained but also to transverse forces which are elicited by longitudinal

strain. If the longitudinal strain is removed, these forces pull in a transverse direction and help restore the original situation. Since these forces are moreover subject to the laws of viscoelasticity, the fiber bundles elongated by the longitudinal strain may become shorter than they had originally been if the transverse forces continue to act in a retardant manner (see below).

Hysteresis. In an ideal elastic tissue, the loading and unloading graphs coincide. If the unloading curve differs from the loading graph, a hysteresis loop is formed. The area within the normalized hysteresis loop is a measure of the energy loss during the load cycle.

Relaxation. The specimens are elongated to the strain levels mentioned above. The elongation is kept constant. The stress that is necessary to achieve this elongation decreases as a result of internal structural dislocations. This decrease can be measured as a function of time (time constant tau) or by the normalized difference between the initial and final values of stress (kappa), respectively.

Retardation. In contrast to the relaxation test, the stress is kept constant in the retardation test if the selected strain level is reached. Since structural dislocations induced by stress are slow to develop against friction, elongation continues to progress, even if the stress remains constant. Retardation can also be measured by a time constant (tau) and the normalized difference between the initial and final value of strain (kappa).

Inverse Relaxation. The specimen is loaded to a selected strain level, e.g., 5%; it is then deloaded to a lower level, e.g., 2.5%. The stress required to maintain this strain level increases.

Inverse Retardation. The specimen is loaded to a selected strain level, e.g., 5%; it is then deloaded to a lower level, e.g., 2.5%. If the stress is kept constant at this level, the specimen becomes shorter. In our opinion, this is an important observation, since it demonstrates that under certain conditions, a connective tissue sample can become shorter without interference by external or cellular forces.

Recovery Time. If a first loading and unloading test is followed immediately by a second one, the second test differs significantly from the first because of the lasting effect of the first test. For the second test to result in exactly the same graph as the first, it must be performed after a certain time interval. This interval must elapse in order to allow the dislocated structures resulting from the first test to return to the original configuration. This is the recovery time, which is measured in minutes.

Morphology

We distinguished the following morphologic classes:

Normal Palmar Aponeurosis. In NPA, the individual fiber bundles are well defined and the loose tissue between the bundles is transparent. When illuminated from an oblique angle, fiber bundles in a state of relaxation show a cross striation as an expression of the wavy course. With delicate longitudinal stress, the cross striation disappears because the fiber bundles elongate. The cross striation reappears if the stress is removed.

Apparently Normal Palmar Aponeurosis. In patients with DD, some areas of palmar aponeurosis still present normally, especially at the radial side. Under the light microscope, this tissue does not appear different from NPA tissue; the only actual difference is that ANPA tissue is derived from patients with DD.

Thickend Fiber Bundles. Patients with DD display thickened fiber bundles (THFB) still showing the original bundle structure; the cross striation, however, has disappeared. Apparently, the fiber bundles are already in a relaxed state when the finger is exposed to a certain stress. The light microscope reveals THFB due to increased collagen synthesis. This is the result of the activity of the *local* fibroblasts, because at this stage there is no fibroblast proliferation whatsoever. THFB do not contain elastic fibers (EF). In the loose connective tissue (LCT) between fiber bundles, however, there are accumulations of thick and fragmented EF. Due to the thickening of individual fiber bundles (FB), the epi- or peritenonal tissue is compressed and disappears, and the THFB fuse to larger units.

Contracture Bands. These bands consist of thickend and fused FB. At some locations there are fusiform enlargements. The original structure of the FB has disappeared except for traces, and at this stage the tissue shrinks and contractures develop. Microscopically, there are nodules of cellular proliferation consisting mainly of fibroblasts, which originate from perivascular spaces. These cells break down the preexisting collagen framework and produce new collagen in patterns resembling vascular structures rather than corresponding to functional requirements. CB tissue contains a higher percentage of type III collagen, which is produced during wound healing and scar formation. In some areas, the cells are similar to secretory fibroblasts and continue to produce collagen; other fibroblasts resemble myofibroblasts. In later stages, the collagen content increases and the number of cells is reduced to a few irregularly distributed accumulations with a scar-like appearance. CB do not contain EF.

Material and Methods

The study was performed in two phases. In the first phase we studied specimens obtained from patients operated upon for carpal tunnel syndrome (n = 23) as NPA (classified as group 1). Group 2 (n = 13) consisted of specimens from patients with DD matching the definition of ANPA. In group 3 (n = 11) we examined specimens with THFB. Group 4 (n = 13) consisted of specimens of CB not showing the scar-like residual stage. In group 5 (n = 5) there were specimens of CB showing the scar-like residual stage. The number of specimens in the second study are given in each table.

Mechanical Tests

Uniaxial strain-controlled tensile tests were performed at a rate of 1% per minute. Strain was defined as the alteration of length per original length of specimens. Displacements were measured with a potentiometer, at an open length of 100 mm and a resolution of 10 μm. Loads were recorded with a load cell, at a maximum load of 100 N and a resolution of 10 mN.

In the case of hysteresis tests, the unloading phase immediately follows the loading procedure. From the load cycle we obtained the residual strain, i.e., the strain remaining after complete removal of the load, and the normalized hysteresis loop, i.e., the ratio of the area between the loading and unloading graph to the area below the loading graph, which represents the strain energy. The normalized hysteresis loop is a measure of the energy loss during the load cycle.

Stress relaxation was conducted at constant strain levels of 2.5%, 5.0%, and 7.5%. During relaxation the stress required to keep strain constant decreases as a function of time. The resulting relaxation graph can be described in terms of the time constant (tau) and the normalized difference between the initial and final stress values. The time constant is defined as the inverse of the initial slope. The normalized difference between the initial and final stress values represents the viscous stress component. On the other hand, if strain is kept constant after partial deloading of a sample, stress increases as a function of time. This phenomenon is called inverse relaxation.

Retardation or creep tests were performed starting from the following initial strain values: 2.5%, 5.0%, and 7.5%. After achieving these strain levels, the load was kept constant, and the strain was plotted as a function of time. Analogous to the relaxation graph, the retardation graph can be characterized by a time constant. The viscous component of stress is defined according to the increase of strain during retardation as the normalized difference between the initial and final strain values. If stress is kept constant after partial deloading of a sample, strain decreases as a function of time. This experimental procedure is called inverse retardation. If load cycles are repeated several times, the loading and unloading graphs of a cycle generally do not coincide with the previous ones. After a certain time interval between load cycles has elapsed, the original biomechanical properties of the specimens can be restored. This time interval is called recovery time.

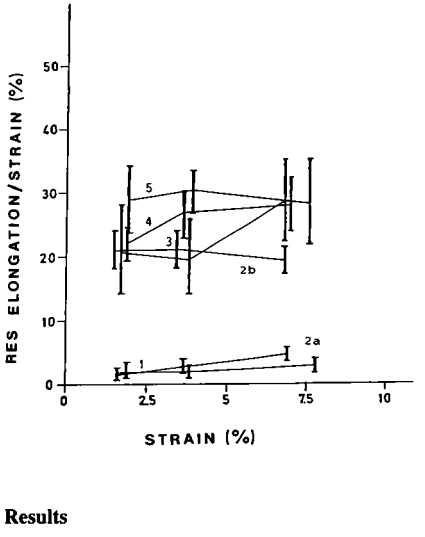


Fig. 1. Normalized residual elongation for three strain levels

Results

First Study

Residual Elongation. Figure 1 illustrates normalized residual elongation for strain levels 2.5%, 5.9%, and 7.5%. It is evident that residual elongation in group 1 was very low (1 in Fig. 1). The residual elongations in groups 3, 4, and 5 (3, 4, 5 in Fig. 1) consisted of 20% to 30%. The 13 patients in group 2 (presenting with ANPA) fell into two different groups. Group 2a was not only normal from the morphologic point of view, it also behaved like NPA patients with respect to residual elongation. Even though they were morphologically normal, however, the tissue obtained from patients in group 2b displayed biomechanical behavior that was similar to that of the tissue obtained from the pathologic groups.

Mechanical Relaxation. Figure 2a presents the initial slope of the relaxation curve for different strain levels (2.5%, 5.0%, and 7.5%). With a 2.5% strain level, there was a significant difference between group 1 (NPA) and groups 3, 4, and 5. Group 2 was in the middle between the two extremes. With a 5% strain level, the values for groups 1 and 2 came closer to those for the pathologic tissue; at a 7.5% strain level, the difference was minimal. If one analyzes the patients in group 2, two characteristic subgroups can be distinguished (Fig. 2b). Some of them behaved more like the NPA specimens (2a in Fig. 2b), and others were more similar to the pathologic tissue (2b in Fig. 2b). The same was true of the viscous fraction (kappa) in the relaxation

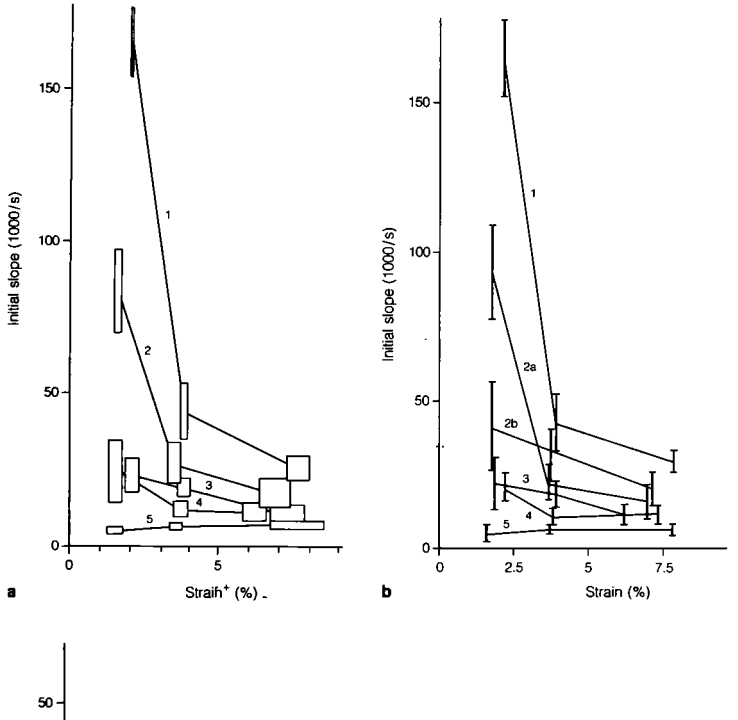


Fig. 2. **a** Initial slopes of relaxation curves for three strain levels for the five groups of patients. **b**, **c** Values for group 2 for initial slope and for viscous fraction divided into two subgroups

Table 1a,b. Residual strain

a Strain levels for unloading phase

Tissue	(*n*)	2.5%	5.0%	10.0%
NPA	23	0.22 +/− 0.13	0.18 +/− 0.07	0.89 +/− 0.08
ANPA	13	0.30 +/− 0.15	0.50 +/− 0.20	0.80 +/− 0.20
THFB	16	0.50 +/− 0.08	1.50 +/− 0.30	3.00 +/− 0.70
CB	13	0.70 +/− 0.20	1.50 +/− 0.20	3.10 +/− 0.50

b Levels of significance

p	NPA	ANPA	THFB	CB
NPA	–	ns	0.05	0.05
ANPA	ns	–	ns	0.05
THFB	0.05	ns	–	ns
CB	0.05	0.05	ns	–

Table 2a,b. Hysteresis loop

a Strain levels for unloading phase

Tissue	(*n*)	2.5%	5.0%	10.0%
NPA	23	5.0 +/− 0.8	5.0 +/− 1.0	8.2 +/− 1.3
ANPA	13	7.0 +/− 2.0	10.0 +/− 3.0	15.0 +/− 5.0
THFB	16	16.0 +/− 2.0	16.0 +/− 1.0	21.0 +/− 2.0
CB	13	19.0 +/− 3.0	21.0 +/− 4.0	30.0 +/− 2.0

b Levels of significance

p	NPA	ANPA	THFB	CB
NPA	–	ns	0.01	0.01
ANPA	ns	–	0.01	0.01
THFB	0.01	0.01	–	ns
CB	0.01	0.01	ns	–

test (Fig. 2c). It is interesting to note that changes in biomechanical properties preceded morphologic changes in light microscopic studies.

Second Study

Residual Elongation. Table 1 represents the strain level for the unloading phase of NPA, ANPA, THFB, and CB. The numbers of the specimens are given under *n*. The values are proportional to strain levels of 2.5%, 5.0%, and 10.0%. Table 1b presents the levels of significance. As in the first study, there was a difference between NPA and ANPA; in this series, however, the difference is not significant. The values for THFB differed significantly from those for NPA. While they were much higher than the values for ANPA, this

Table 3a,b. Relaxation time

a Strain levels for relaxation phase

Tissue	(*n*)	2.5%	5.0%	10.0%
NPA	19	6.1 +/− 0.4	22.6 +/− 4.8	39.4 +/− 6.2
ANPA	11	12.0 +/− 2.0	36.8 +/− 7.3	57.8 +/− 15
THFB	11	40.0 +/− 15	82.0 +/− 20	95.0 +/− 32
CB	5	196 +/− 39	156 +/− 26	154 +/− 38

b Levels of significance

p	NPA	ANPA	THFB	CB
NPA	–	0.05	0.01	0.01
ANPA	0.05	–	ns	0.01
THFB	0.01	ns	–	0.01
CB	0.01	0.01	0.01	–

Table 4a,b. Retardation time

a Initial strain levels

Tissue	(*n*)	2.5%	5.0%	10.0%
NPA	20	7.9 +/− 0.9	44.0 +/− 8.7	47.0 +/− 3.8
ANPA	13	11.0 +/− 1.2	43.0 +/− 7.9	80.0 +/− 6.9
THFB	5	15.0 +/− 1.3	53.0 +/− 9.2	80.0 +/− 5.4
CB	8	45.0 +/− 8.2	77.0 +/− 14	77.0 +/− 8.3

b Levels of significance

p	NPA	ANPA	THFB	CB
NPA	–	0.05	0.01	0.01
ANPA	0.05	–	0.05	0.01
THFB	0.01	0.05	–	ns
CB	0.01	0.01	ns	–

difference was not statistically significant, however. The values for CB differed from those for NPA and ANPA, but not from those for THFB.

Hysteresis. NPA and ANPA do not differ significantly with respect to hysteresis. There is a clear significant difference between the two groups and THFB and CB specimens, however (Tables 2a and 2b).

Relaxation Time. The values for NPA and CB specimens at various strain levels are shown in Tables 3a and 3b. There is a strong difference between NPA and ANPA at 2.5% strain. The results of this test confirm the results of the first study with respect to the difference between NPA and ANPA.

Retardation Time. Values for retardation time are presented in Table 4a, and levels of significance for a strain level of 2.5% are provided by Table 4b. There

Table 5a,b. Inverse relaxation

a Values obtained

Tissue	(*n*)	tau	kappa
NT	7	4.2 +/− 1.0	0.03 +/− 0.02
NPA	9	6.3 +/− 1.7	0.62 +/− 0.40
ANPA	8	12.0 +/− 2.6	2.40 +/− 0.80
THFB	5	7.1 +/− 1.4	6.20 +/− 2.90
CB	8	18.8 +/− 5.0	11.80 +/− 4.20

b Levels of significance

p (tau)	NT	NPA	ANPA	THFB	CB
NT	–	ns	0.01	ns	0.005
NPA	ns	–	ns	ns	0.005
ANPA	0.01	ns	–	ns	ns
THFB	ns	ns	ns	–	0.005
CB	0.005	0.005	ns	0.005	–

p (kappa)	NT	NPA	ANPA	THFB	CB
NT	–	ns	ns	0.005	0.001
NPA	ns	–	ns	0.005	0.001
ANPA	ns	ns	–	0.005	0.001
THFB	0.005	0.005	0.005	–	ns
CB	0.001	0.001	0.001	ns	–

was a significant difference between NPA and ANPA as well as between these and other groups. CB and THFB were the only groups that did not differ significantly. This again confirms the results of the first study.

Inverse Relaxation. Table 5a provides the values for inverse relaxation time (tau) and the viscous fraction obtained by the inverse relaxation test (kappa). Table 5b shows the difference in levels of significance between the groups for both tau and kappa for loading to 5% and deloading to 2.5% strain. There was no significant difference between NPA and ANPA; both groups differed significantly from CB for tau and THFB for kappa, however.

Inverse Retardation. Table 6a demonstrates inverse retardation time (tau) and the viscous fraction obtained by the inverse retardation test (kappa). There was no significant difference between NPA and ANPA specimens; THFB and CB specimens clearly differed, however.

Mechanical Recovery. Figure 3 shows the recovery time with a load strain test investigating a specimen of NPA. After ten minutes the loading curve once again resembled the original one. Figure 4 represents a typical test of a CB specimen. Even after 420 min, the loading curve still differed from the original one. Mechanical recovery values in minutes for the different groups, including specimens with normal tendons, are given in Fig. 5. There was already a

Table 6a,b. Inverse retardation

a Values obtained

Tissue	(*n*)	tau	kappa
NT	9	217 +/− 45	6.4 +/− 1.3
NPA	9	143 +/− 27	3.3 +/− 0.6
ANPA	3	222 +/− 17	9.2 +/− 0.4
THFB	4	489 +/− 59	23.8 +/− 2.8
CB	9	1050 +/− 234	43.2 +/− 3.9

b Levels of significance

p (tau)	NT	NPA	ANPA	THFB	CB
NT	–	ns	ns	0.01	0.005
NPA	ns	–	ns	0.005	0.005
ANPA	ns	ns	–	0.005	0.005
THFB	0.01	0.005	0.005	–	ns
CB	0.005	0.005	0.005	ns	–

p (kappa)	NT	NPA	ANPA	THFB	CB
NT	–	ns	ns	0.001	0.001
NPA	ns	–	ns	0.001	0.001
ANPA	ns	ns	–	0.001	0.001
THFB	0.001	0.001	0.001	–	ns
CB	0.001	0.001	0.001	ns	–

Table 7a,b. Mechanical recovery

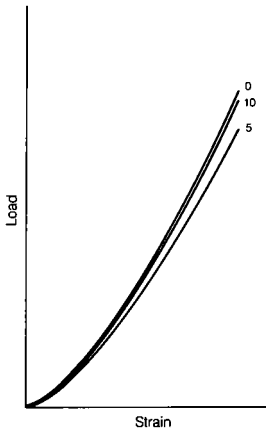
a Values

Tissue	(*n*)	Interval (min)
NPA	9	12.2 +/− 1.2
ANPA	8	21.3 +/− 1.3
THFB	5	45.0 +/− 8.7
CB	8	210 +/− 45

b Levels of significance

p (interval)	NPA	ANPA	THFB	CB
NPA	–	ns	0.005	0.005
ANPA	ns	–	0.01	0.01
THFB	0.005	0.01	–	0.05
CB	0.005	0.01	0.05	–

difference in recovery time between NPA (12.2 +/− 1.2) versus ANPA (21.3 +/− 1.3), but this is not significant. There was a much bigger difference compared to THFB. The striking increase in recovery time is shown by the difference between CB and the other groups (Fig. 5), however.



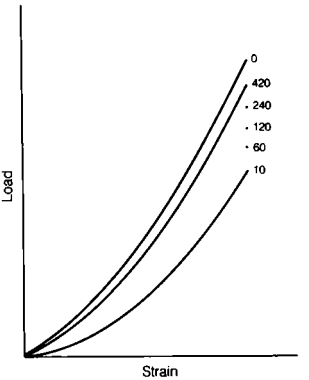


Fig. 3 *(left)*. Recovery time with load strain test for normal palmar aponeurosis

Fig. 4 *(right)*. Loading curves for contracture band recovery after different periods of time

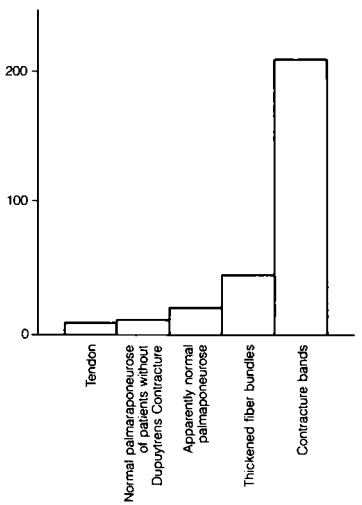


Fig. 5. Recovery period (in min) for the different groups

Discussion

In NPA specimens, the residual strain was rather uniformly small. It increased in some of the ANPA and significantly increased in THFB and CB specimens. The return to zero after loading with elongation was certainly supported by EF. We may therefore assume that EF functioning was less than optimal in these patients. A quantitative study would be necessary to prove this observation. It is very difficult to explain differences in relaxation times between these groups. Inverse relaxation arises in the transient zone between ANPA and THFB. Thickening of the fiber bundles accompanied by fusion and loss of the peri- and epitenonal structures may be the morphologic background for this change in mechanical properties. Prolonged mechanical recovery times are linked to specimens which are in the process of developing contracture. It will be our next task to analyze these results in the light of our clinical data.

Summary

The mechanical properties of different specimens obtained from patients with DD and of palmar aponeurosis specimens obtained from patients without DD who are suffering from carpal tunnel syndrome have been studied. The residual elongation after loading and unloading to 2.5% strain increased in some cases of ANPA in patients with DD. The same was true for the relaxation time. This means that these particular mechanical changes precede the typical morphology. Inverse relaxation and inverse retardation increase if the structure of the fiber bundle changes. The mechanical recovery time is strongly linked to the process of contracture.