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Research Article

The Biomechanical Properties of Human Fresh-Frozen vs Thiel Embalmed Foot Tendons

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Abstract

Background: The aim of this study was to directly compare the effects of Thiel embalming on the mechanical properties of three human tendons to similar tendons from the fresh-frozen, ipsilateral foot of the same cadavers.

Method: Following pre-conditioning, biomechanical tensile tests were conducted on tibialis anterior, peroneus longus and Achilles tendons at a strain rate of 5 mm/minute, using an optical extensometer for the direct measurement of sample strain.

Results: Qualitatively, all tested tendons exhibited similar mechanical behaviour characterised by an initial toe region followed by a region of linear behaviour. Quantitatively, however, averaging the results for the three cadaveric samples revealed consistent differences across all tendons in the case of unloading stiffness.

Conclusion: Thiel embalming method is suitable for preservation of human tendons, with embalmed tendons appearing to have similar mechanical behaviour compared to their fresh frozen counterparts. Although quantitative differences do exist, these do not disprove the use of Thiel embalmed tendons in comparative studies once care is exercised when utilizing these for biomechanical testing. However, *in vitro* results used to infer *in vivo* function should always be treated with caution.

Keywords: Biomechanical Properties; Human Fresh-Frozen; Thiel Embalmed Foot Tendons

Introduction

In humans, testing for the mechanical properties of tendons is often performed in cadaveric fresh-frozen samples. The use of these samples, which are stored at -20°C until they can undergo testing, is considered as the gold-standard in this type of research.

The use of fresh-frozen samples, however, has two very important limitations: thawed tissue has a limited time period during which testing can be performed, thus placing considerable time-constraints and pressure on the investigator, consequently raising the possibilities of human error during testing which could adversely affect results. Secondly, freezing only delays the decaying

process and once thawed, the risk of possible infection for the investigators is increased [1].

In order to overcome these limitations, several preservation techniques including formalin fixation, Thiel-fixation, and alcohol-glycerine fixation, are reported [1]. Thiel fixation has been claimed to reduce risks of infection whilst maintaining tissue suppleness for a long period of time without the need for refrigeration, thus leaving the tissue looking 'normal'. The colour, consistency and transparency of the tissue are thus very well preserved [2].

This 'soft-fix' method has become increasingly popular in anatomy laboratories; however their use in biomechanical testing is rather limited due to a lack of scientific data arising out of appropriate studies. The importance of determining the mechanical characteristics of these tendons becomes evident when one considers that these Thiel embalmed specimens have the potential to be utilised in biomechanical testing, for example following the development of new surgical techniques. Although there appears to be general consensus that Thiel embalmed soft tissue may be 22 - 45% more flexible [3] than fresh-frozen specimens, possibly due to dis-organisation of muscle fibre following considerable fragmentation of the muscle proteins [4], few studies have been conducted to confirm, or otherwise, this hypothesis.

To date, there is a paucity of evidence as to whether this method of embalming leaves tendons with appropriate mechanical characteristics that enable them to be utilized for biomechanical testing, with very few studies on individual Thiel embalmed tendons having been conducted. Fessel., *et al.* [5], who investigated the digitorum profundus and rat-tail tendons, which demonstrated a lower failure stress when compared with their fresh-frozen counterparts, whilst Liao., *et al.* [6] mechanically tested human peroneus brevis and peroneus longus tendons, report that Thiel embalming preserves human tissue elasticity. Both these studies have some limitations; while the former compares only 1 type of human tendon, the latter study's two tendons' characteristics from 3 cadavers were compared with data from the literature. Another study on 7 human Achilles tendons reports that Thiel embalming significantly alters the Young's modulus of human tissue [7].

Aim of the Study

The aim of this study was to quantify and directly compare the effects of Thiel embalming on the mechanical properties of three human tendons. Throughout this study, the Thiel embalmed tendons were directly compared to similar tendons from the ipsilateral foot of the same cadavers following a method of preservation specifically developed for this study that has never been previously reported in literature.

Methods

This research was approved by the University Research and Ethics Committee of the University of Malta prior to data collection. Human tissue utilized throughout this study were received and stored at the Anatomy Department at the same University, following strict guidelines as outlined within the local legislation that strictly followed the European Union directives pertaining to the handling of human tissues.

The feet from three cadavers voluntarily donated for scientific research were utilised throughout the study. A technique for the fresh-frozen preservation of one foot, and Thiel embalming of the other, was developed specifically for this study as follows. Prior to preservation, each cadaver had a single leg amputation 20 cm proximal to the medial malleolus, which was then maintained in a frozen state at -20°C. Each cadaver was then maximally perfused through the femoral artery with Thiel embalming fluid through a plastic tube inserted into the proximal and distal segments of the femoral artery sectioned just below the inguinal ligament. A second tube was inserted into the femoral vein at the same level and left to drain into the embalming table sump. Saline was pumped into the cadaver until the fluid which ran out of the venous drain was free of blood. Thiel solution was then injected into the cadaver. External embalming was carried out in a tank.

Visual inspections for significant differences between the right and left foot of each cadaver was carried out to ensure similarity. Signs of significant deformity and past surgery would have rendered these specimens unusable.

Before testing, the fresh-frozen samples were allowed to thaw for 24 hours prior to dissection. The embalmed feet, on the other hand, had no need for any specific preparation. The tendons of the tibialis anterior, peroneus longus, together with the Achilles tendon, were harvested by blunt dissection. The same part of the tendon was prepared for testing for each specimen.

All testing was performed in the same laboratory, at 18°C and 57% humidity. Each end of the tendon was wrapped in a thin piece of cloth and then mounted on the load frame using purpose-built compression grips as per Fessel., *et al* [5]. Consequently, due diligence was maintained when tightening these clamps in order not to damage the tendons.

These clamps, in turn, had previously been mounted on both ends of a Testometric (Lincoln, United Kingdom) M350-20CT materials testing device, which had a 1000N load cell (type DBBMTCL).

Each tendon was marked with an indelible marker to facilitate optical referencing [8] for a two-camera Zwick extensometer, calibrated as per manufacturer instructions, thus providing markers that facilitated the direct measurement of sample strain (Figure 1).

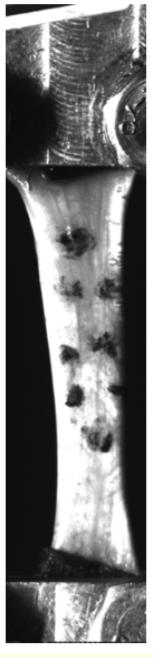

Figure 1: Fresh frozen tibialis posterior tendon undergoing testing in the tensile testing machine.

Pre-conditioning of the tendon was performed by applying a tensile load up to 10N, held for 30 seconds and released back to 0N, at a load rate of 0.25 N/sec. This was repeated for 10 times to ensure the comparative results are not influenced by the loading history of the samples [8,9].

After preconditioning the samples were subjected to one load/unload cycle of tensile loading to a maximum force of 100 N. Both loading and unloading were performed by imposing a constant displacement rate (grip-to-grip) of 5 mm/min.

After synchronisation force data exported from the load frame and strain data from the video extensometer were used to draw the force/strain graphs for each sample and to measure their maximum strain (i.e. strain for 100 N tension force), hysteresis ratio and stiffness for loading and unloading. Hysteresis ratio was calculated by subtracting the area below the force/ strain graph for unloading from the respective area for loading and divided by the area below loading. Stiffness for loading and unloading were calculated as the slope of the force/strain graph for force between 80 N and 100 N for the loading and unloading segments respectively. A paired sample t-test was also performed using Microsoft Excel to assess the statistical significance of the differences found between fresh frozen and Thiel embalmed samples.

Results

Nine tendons (3 fresh frozen and 3 Thiel-embalmed) from 3 cadavers (2 females, 1 male, mean age 84 years) were utilized throughout this study.

Eight out of the nine tests were completed successfully. An error in data collection during the testing of tibialis anterior sample #2 led to its elimination from the rest of the analysis (Figure 2).

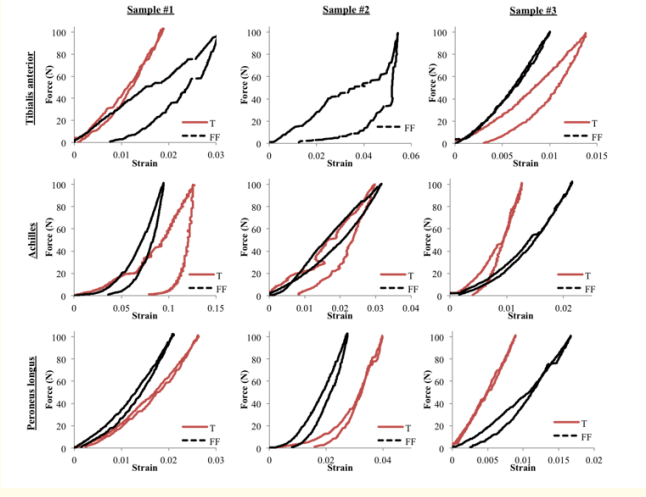

Figure 2: The force/strain graphs for all pairs of Thiel embalmed (T) and fresh frozen (FF) samples of tibialis anterior (first row), Achilles (second row) and peroneus longus (third row) tendons included in this analysis. Results for tendons harvested from cadaveric sample 1, 2 and 3 are shown in first, second and third column respectively. The graph for the fresh frozen tibialis anterior sample #2 is also shown for completeness.

A qualitative comparison between the force/ strain graphs of Thiel embalmed tendons and their fresh frozen counterparts indi-

cates that all tested tendons exhibit similar mechanical behaviour characterised by an initial toe region followed by a region of linear behaviour (Figure 2). In quantitative terms, averaging the results for the three cadaveric samples revealed consistent differences across all tendons in the case of unloading stiffness (Table 1). More specifically unloading stiffness for Thiel embalmed specimens was 40%, 62% and 14% higher than the stiffness of their fresh frozen counterparts for tibialis anterior, Achilles and peroneus longus tendon respectively. Paired samples t-test showed that the aforementioned difference was statistically significant in the case of Achilles tendon (Table 1).

Sample			**#1**	**#2**	**#3**	**Average**	**STDEV**	**P-value**
Age (y)			**84**	**83**	**85**	**84**		**-**
Sex (M/F)			**Female**	**Female**	**Male**	**-**	**-**	**-**
Max strain	Tibialis anterior	T	0.019	-	0.013	0.016	0.004	-
		FF	0.030	0.054	0.010	0.020	0.014	
	Achilles	T	0.127	0.030	0.013	0.057	0.062	0.62
		FF	0.094	0.032	0.021	0.049	0.039	
	Peroneus longus	T	0.026	0.040	0.008	0.024	0.016	0.69
		FF	0.021	0.028	0.017	0.022	0.005	
Hysteresis ratio	Tibialis anterior	T	0.19	-	0.38	0.28	0.13	-
		FF	0.49	0.70	0.02	0.25	0.33	
	Achilles	T	0.81	0.16	0.21	0.39	0.36	0.30
		FF	0.42	0.18	0.09	0.23	0.17	
	Peroneus longus	T	0.09	0.18	0.01	0.09	0.08	<u>0.04</u>
		FF	0.16	0.31	0.16	0.21	0.08	
Stiffness Loading (N)	Tibialis anterior	T	6901	-	8765	7833	1318	-
		FF	3327	2542	15897	9612	8888	
	Achilles	T	1550	3999	15712	7087	7569	0.43
		FF	2353	3205	7495	4351	2756	
	Peroneus longus	T	5931	6623	11820	8125	3219	0.64
		FF	6596	7137	8538	7424	1002	
Stiffness Unloading (N)	Tibialis anterior	T	7932	-	19141	13536	7925	-
		FF	6869	16136	12447	9658	3944	
	Achilles	T	7625	7283	13169	9359	3304	<u>0.03</u>
		FF	4458	4588	8308	5785	2186	
	Peroneus longus	T	7644	11070	13900	10871	3133	0.58
		FF	8981	11095	8357	9478	1435	

Table 1: The experimental results for the Thiel embalmed (T) and fresh frozen (FF) tendons from all three cadaveric samples included in this study. The P-values calculated using paired samples t-test are also presented for all tendons with the exception of tibialis anterior. P-values lower than 0.05 are underlined to highlight statistically significant differences between Thiel embalmed and fresh frozen samples.

Paired samples t-test also revealed statistically significant differences in terms of hysteresis ratio in the case of peroneus longus, with Thiel embalmed specimens having 56% lower average hysteresis ratio compared to their fresh frozen counterparts (Table 1). In contrast to the results for peroneus longus average hysteresis ratios for Thiel embalmed tibialis anterior and Achilles tendons ap-

peared to be 12% and 73% higher compared to their fresh frozen counterparts respectively. However, the aforementioned differences were not statistically significant ($p > 0.05$).

Discussion

This study differed significantly from the few previous studies that compared Thiel to fresh frozen samples in the method employed in order to obtain samples from the same cadaver, which could consequently allow for direct comparison. Furthermore, this study has utilized the most number of tendons for direct comparison.

Although it is recognized that the two feet of the same person are not necessarily identical, the use of contralateral feet in a set ensured that these paired feet would have been subjected to the same number of loading cycles in their lifetime. Also, the possibility of tendons being affected by disease would be similar to each foot.

One of the most challenging aspects of tendon mechanical testing is rigidly gripping the samples without damaging them or altering their mechanical properties [10,11]. According to literature excessive compression and the use of friction based grips is likely to cause damage to the tissue itself, whilst the use of freeze-clamps with liquid nitrogen can significantly affect the mechanical behaviour of the samples if the freezing areas are not properly controlled [10,11]. Considering the challenges associated with freezing-clamps it was decided that their use would complicate the experiment beyond that merited by the scope of the research in the first place. Moreover, since the aim of the study was of a comparative nature which did not include the yield point allowed performing the tests at a relatively low tension force (i.e. 100 N). This enabled rigidly gripping the samples without the need for substantial compression of the samples within the grips minimising the risk of damage. The effect of possible slippage of the samples out of the grips was eliminated by directly measuring tendon elongation using optical extensometer.

Conclusion

In qualitative terms Thiel embalmed tendons appeared to have similar mechanical behaviour compared to their fresh frozen counterparts, however quantitative differences do exist.

The differences found do not disprove the use of Thiel embalmed tendons in comparative studies.

In any case *in vitro* results used to infer *in vivo* function should always be treated with caution.

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