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## Biomechanical quantification of graft preconditioning

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Meyer, Dominik C.; [Snedeker, Jess Gerrit](#) ; Weinert-Aplin, Robert A.; Farshad, Mazda

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# Viscoelastic adaptation of tendon graft material to compression: biomechanical quantification of graft preconditioning

Dominik C. Meyer · Jess G. Snedeker ·  
Robert A. Weinert-Aplin · Mazda Farshad

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## Abstract

**Purpose** The tensile viscoelastic behaviour of tendon tissue is of central biomechanical importance and well examined. However, the viscoelastic tendon adaptation to external compression, such as when a tendon graft is fixated with an interference screw, has not been investigated before. Here, we quantify this adaptive behaviour in order to develop a new method to mechanically precondition tendon grafts and to better understand volumetric changes of tendinous tissue. The hypothesis of this study was that under compressive loads, tendon grafts will undergo a temporary volumetric (and therefore diametric) reduction, due to the extrusion of water from the tendon.

**Methods** Compressive testing was performed on a material testing machine and load applied through the use of a custom-made mould, with a semi-circular cross section to accommodate the tendon graft. The effects of different compressive forces on the length, diameter and weight of tendon grafts were measured by calipers and a weighing scale, respectively. Further, different strain rates (1 vs. 10 mm/min) ( $n = 6$ , per rate), compression method (steady compression vs. creep) ( $n = 15$  for each method) and different compression durations (1, 5, 10 min) ( $n = 5$  for each duration) were tested to identify the most effective combination to reduce graft size by preserving its macroscopic structure.

**Results** The effect of compression on volume reduction (75 % of initial volume and weight) reached a plateau at 6,000 N on an 8-mm tendon bundle. Length thereby increased by approximately 10 %. Both steady compression

and creeping were able to reduce dimensions of the graft; however, creeping was more effective. There was no difference in effect with different durations for compression ( $p > 0.05$ ) in both methods.

**Conclusion** The viscoelastic behaviour of hamstring tendon grafts under pressure allows preconditioning of the grafts for reduction of volume and diameter and therefore to drill a smaller bone tunnel, retaining more of the original bone. At the same time, the collagen content of the transplant is preserved and a tight fit of the transplant in the bone tunnel achieved.

**Keywords** Viscoelastic tendon adaptation · Compression · Interference screw

## Introduction

The tensile elastic and viscoelastic properties of tendons are of great importance for the function of the musculotendinous unit and have been extensively investigated [7, 15–18]. When used as an auto- or allograft, however, tendon material will not only be subjected to tensile, but also to compressive force, such as in a tunnel fixated with an interference screw, within the loop of a suture stitch or if bent around a bone edge [11]. The behaviour of tendon tissue under compressive load has been described before [5, 10], however it has not yet been quantified in detail. As we could observe in an earlier study that tendon tends to slowly adapt to external compressive force [11], we speculated that under pressure, water would be pressed out from tendon tissue and tendon volume lost. We further speculated that it might be useful to exploit this effect before graft implantation by compressing the tendon transplants to a desired shape and to anticipate later loss of volume and undesired loss of geometric fit [6, 12].

D. C. Meyer · J. G. Snedeker · R. A. Weinert-Aplin ·  
M. Farshad (✉)  
Department of Orthopedics, University of Zürich,  
Balgrist, Forchstrasse 340, 8008 Zürich, Switzerland  
e-mail: mazda.farshad@balgrist.ch

**Table 1** Material used for different test settings

Test purpose	Tendon type	<i>n</i>
Volume reduction in relation to the compressive force	Sheep extensor digitorum tendons	13
Effect of strain rate (10 and 1 mm/min)	Calf flexor digitorum tendons	12 (6 per strain rate)
Effect of relaxation time (1, 5 and 10 min)	Calf flexor digitorum tendons	15 (5 per hold time)
Effect of creeping (20, 75, 150 cycles)	Calf flexor digitorum tendons	15 (5 per group)

The hypothesis of this study was that under compressive loads, bundled tendon grafts will undergo a temporary volumetric (and therefore diametric) reduction, due to the extrusion of water from the tendon.

Therefore, the aim of this study was to quantify the viscoelastic and volumetric adaptation of tendon tissue under various pressurizing conditions and to translate the findings into a practical surgical method for tendon compression by the example of tendon bundles, such as those used for reconstruction of cruciate ligaments of the knee. The final clinical relevance is to achieve a smaller drill hole for the same graft and tighter fit with better ingrowth, characterized by less or no tunnel widening.

## Methods

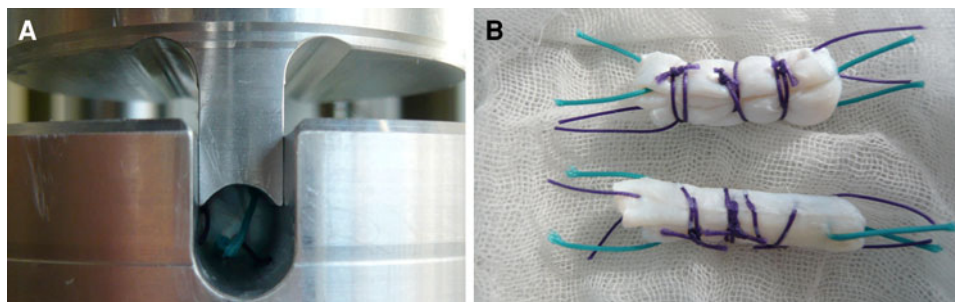
Fresh flexor digitorum tendons of the calf and adult sheep extensor digitorum tendons were used (Table 1). Due to better availability, further experiments with need of large amount of tendons were performed with calf flexor digitorum tendons. Preliminary experiments were performed to assure the interspecies similarity with regard to behaviour of the tendon material under compression [3].

Tendons were harvested directly after slaughter and additional tissue was removed. The number of folds of tendon required to obtain grafts of 8- to 9-mm diameter and 30-mm length was determined by measurement with a caliper and verified by use of a pull-through block, with 7-, 8- and 9-mm holes as a rough check of graft diameter. The

diameter was measured at the ends and mid-length of the tendon, and the mean of the three values was considered to be a representative diameter of the graft. The tendon was laid out in a zigzag pattern, with Vicryl® USP 0 at the ends of the tendon and Ethibond® sutures (Ethicon; Johnson&Johnson, Norderstedt Germany) were placed at the folds of the tendon. Once all the sutures were in place, the folds of the tendon were lightly pushed together, so that there were no sections of the graft protruding out. With the bundles still under tension, No. 0 Vicryl sutures were used to pierce the tendon about 1/4 of the way along the graft and loop the suture around the entire graft. This was repeated at halfway and 3/4 of the length of the graft to finish the bundling process. Finished grafts were measured with a caliper (with a tolerance of 0.05 mm) to determine the final length and diameter and weighed on a weighing scale (with a tolerance of 0.005 g), before being stored in Ringer's lactate-soaked gauze.

In order to characterize the behaviour of tendon in compression, an aluminium custom mould was manufactured, which consisted of a loading head and a mould which would accommodate a circular section of material in compression (Fig. 1). All mechanical testing was performed on a universal testing machine (Zwick 1456, Zwick GmbH, Ulm, Germany) with a 20-kN load cell.

Compression was applied by displacement-controlled motion of the load head. Compression of samples was done by initially applying a 5-N preload at a rate of 10 mm/min, at which point the sample could be shifted in the mould, to ensure that the entire sample was compressed by the load



**Fig. 1** An aluminium custom mould with a loading head and a mould (a), which could accommodate a circular section of material in compression, was designed to suit a mechanical testing machine (Zwick 1456, Zwick GmbH, Ulm, Germany) for load- and/or strain-controlled

compression of the grafts. A compressed tendon graft (b, lower graft) showed a smaller diameter and increased total length compared to an untreated graft (b, upper graft)

head. Then, a 100-N nominal load was applied to allow for the bundles to reorganize within the graft and to allow for a more steady compression of the graft up to peak load, as the slippage of bundles across their surfaces would lead to an unsteady loading profile.

#### Volume reduction in relation to the compressive force

Experiments for characterization of the effect of compression on volume were performed on 13 sheep extensor digitorum tendons, which were bundled to become a bundle with a diameter of 8 mm and a length of 30 mm. To determine the geometric changes due to compression at different peak loads in steps of 2,000 N, sample weight, length, volume and diameter were measured before and after compression. Two or three grafts were tested at each compressive load for repeatability. However, to determine the reduction in diameter, the weight loss of the tendon and the compressed graft length were used as a secondary measure to determine the compressed graft diameter.

#### Strain rate

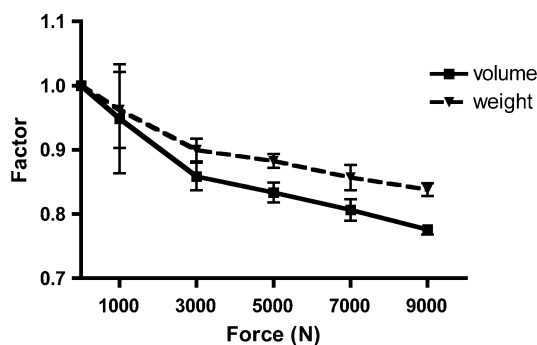
Compression tests to determine an appropriate strain rate were performed at 10 and 1 mm/min, to determine how stable the tendon was under the different rates of loading. Six grafts were tested per group.

#### Relaxation tests

Graft dimensions were taken immediately before and after loading. Grafts were preloaded in the above-explained manner. Load was then increased to 6,000 N at 1 mm/min and held at a fixed displacement for the desired time (1, 5 or 10 min), after which samples were unloaded. Five grafts were tested at each hold time.

#### Effective creep tests

Creep testing was also performed, as this was thought to be the most efficient way of squeezing all of the water out of the tendon, leaving only an elastic material. Graft dimensions were taken immediately before and after loading. Grafts were preloaded in the above-explained manner. The creep test required a loading protocol that performed an effective creep test. This entailed loading up to 6,020 N at 1 mm/min and holding for 1 s before decreasing the load to 5,990 N for 1 s. Cycles between these two forces were performed until the desired duration (1, 5 or 10 min) had been achieved, after which grafts were unloaded completely. It should be noted that approximately 20, 75 and 150 cycles corresponded to 1, 5 and 10 min of load-controlled loading, respectively. Five grafts were tested at each hold time.



**Fig. 2** The normalized volume and the weight of the bundled tendon grafts decreased with increasing compression force

#### Statistical analysis

A standard statistical software (PRISM Version 5.01 for Windows; Graphpad) was used for all analyses. Values were documented as mean with their 95 % confidence interval [mean (lower 95 % CI, upper 95 % CI)]. Where applicable, data were tested for normal distribution using the Kolmogorov–Smirnov test and either two-tailed Student’s *t* test or Mann–Whitney test was employed for intergroup comparison for normal and not normal distributed data, respectively.

For intragroup comparison of the effect of compression on the tissue, the Wilcoxon matched pairs test was used. Significance was set at a level of  $p < 0.05$ .

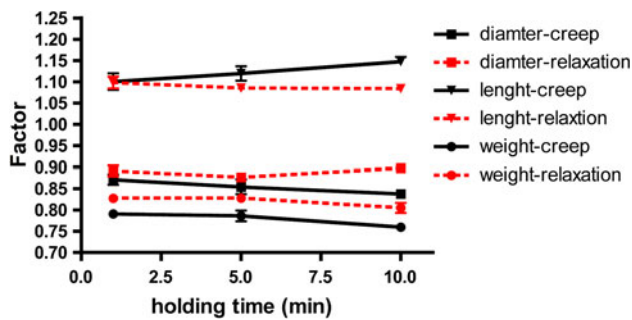
## Results

#### Volume reduction in relation to the compressive force

The volume decreased with increasing compression force (Fig. 2) and reached a plateau regarding the cross-sectional area at about 6,000 N (75 % of initial volume). The decrease of volume was concordant to the decrease in weight (Fig. 2), with a decrease in area and diameter both being constituent parts of the volume.

#### Strain rates

At a strain rate of 10 mm/min of loading up to 6 kN, significant rupturing occurred in four out of four samples, disrupting the transplant partially in the mid-substance. Total disruption did not occur. At a rate of 1 mm/min, no rupturing was observed in any of the cases, with only mild (less than 3 mm) extrusion of tendon from the end of the bundle (and not at the fold of the graft) in two out of four cases.



**Fig. 3** Comparison of the single compression relaxation and creep tests showed the slightly increased diameter reduction and increased gain in length that is possible through the creep approach

### Relaxation tests

The average initial bundle diameters were reduced from 8.3 mm (95 % CI 8.0, 8.7), 8.9 mm (95 % CI 7.8, 10) and 8.7 mm (95 % CI 7.9, 9.5) to 7.4 mm (95 % CI 6.9, 7.9) ( $p = 0.0625$ ), 7.8 mm (95 % CI 6.9, 8.7) ( $p = 0.0625$ ) and 7.8 mm (95 % CI 7.2, 8.3) ( $p = 0.0625$ ) after 1, 5 and 10 min of compression, respectively. Diametric reductions were found to be reasonably constant for relaxation periods between 1 and 10 min ( $p = 0.25$ ).

### Creep tests

The average initial diameters of the bundles used in creep testing were 9.3 mm (95 % CI 0.9, 0.6), 8.3 mm (95 % CI 7.7, 8.9) and 9.3 mm (95 % CI 8.4, 10.2) for 1, 5 and 10 min, respectively, and was reduced to 8.1 mm (95 % CI 7.8, 8.4) ( $p < 0.05$ ), 7.1 mm (95 % CI 6.3, 7.9) ( $p = 0.0625$ ) and 7.8 mm (95 % CI 6.9, 8.7) ( $p < 0.05$ ), respectively, after creep testing. There seemed to be no significant gains in terms of diametric reduction for a creep period of 10 min compared to 1 min ( $p = 0.5$ ). Comparison of the relaxation and creep tests (using values measured directly) showed slightly increased diametric reduction that is possible through the creep approach (Fig. 3).

### Discussion

Tendons are designed to transmit tensile muscle force and react to load in a viscoelastic manner. This has been extensively investigated [7, 14–18] and can in part be attributed to the significant water content, which may exceed 60 % [1] of the total weight. However, tendons also play an important role as grafts for various surgical procedures, particularly for cruciate and collateral ligament repair such as in the knee, elbow and ankle surgery [13]. In almost all of

these reconstructive procedures, and also in common tendon repair, there is the requirement of mechanically holding the graft ends, which is almost invariably achieved by applying a partially compressive force on the tendon tissue [2, 4]. Common fixation techniques include the use of interference screws or sling-shaped suture stitches, creating mechanical hold mostly through pressure-induced friction of the screw or suture against the collagen fibres. However, in a recent study, it has been found that tendon grafts may slowly lose this mechanical fixation due to viscoelastic adaptations in response to the pressure fit created [11]. The water is pressed out from the tendon, which reduces its volume and, consequently, the mechanical fixation of the graft [11].

Even though many operative procedures rely on the compressive resistance of tendon tissue [2, 4], there is no report to our knowledge characterizing the behaviour of tendon tissue under compressive load. The hypothesis of this study was that under compressive loads, bundled tendon grafts will undergo a temporary volumetric (and therefore diametric) reduction, due to the extrusion of water from the tendon.

It was therefore the purpose of this study to understand and quantify the compressive viscoelastic behaviour of hamstring tendon grafts under pressure and consequently to develop a new method for tendon preconditioning to reduce the graft volume before implantation using a dedicated compression device. The motivation is to prevent later viscoelastic loss of pressure and to achieve a tighter fit of the transplant in a smaller bone tunnel and to potentially prevent tunnel widening [12] in case of ACL-replacement.

Limitations of the applied methods are that due to limited availability and large variability of fresh human tendon material, mainly bovine tendons of very similar appearance to human hamstrings were used, which, however, are considered to be comparable to human hamstrings [3]. Due to the large number of different experiments performed, we considered it important not to influence the results additionally with a variability of the tendon quality of unknown extent. In future tests, it will be of interest to study the influence of tendon quality on the behaviour under pressure, and on postoperative swelling in the body.

This study has shown that using external mechanical pressure, the tendon volume may be reduced by 10–30 % through extrusion of water. If we assume a mean water content of 60 % [1] in a normal tendon, this represents an extrusion of 17–50 % of the total tendon water. Interestingly, there was little difference in the volume of extrudable water before mechanical damage to the tendon tissue occurred, regardless of the technique. We speculate that the extruded liquid represents extracellular water, which may be removed relatively easily, while the intracellular water may not be so readily displaced. Even though total volume

was lost, we could document an approximately 5–10 % gain in length of the transplant, which we attributed to an indirect pressure-induced longitudinal elongation and stretching.

These findings seem important in various ways:

Firstly, this method offers a simple approach to decrease the graft volume and to facilitate the handling of a tendon transplant through a process, which anticipates an effect that inevitably occurs, if not during then after implantation. It has been previously shown that modification of the tendon graft surface, namely embossing a screw thread and TCP coating had a large influence on the biomechanical performance of interference screw fixation and resulted in less bone damage inflicted during insertion to a smaller tunnel diameter, while simultaneously achieving superior pull-out strength [5].

Secondly, tendon (or collagenous tissue in general) is under compressive load in virtually any surgical procedure, which utilizes sutures or tendon fixation implants. The here described findings help to better understand and quantify the reaction of the tissue.

Thirdly, the water content of tendon tissue is variable in vivo and influenced by gender, training, inflammation and other factors [8]. Particularly after transplantation, the volume may not only be decreased, but also be increased by 40 % as a reaction to tissue transformation and healing [9]. In future studies, it may be of interest to investigate the volumetric behaviour of compressed transplanted tendons in vivo, or possible treatment options applying pressure to collagenous tissue in vivo.

Fourthly, the observed stretching, which is otherwise attempted through direct pull on the transplant with sutures, may be beneficial for the desired pre-tension of the transplant after implantation.

A secondary observation from this study is that the viscoelastic nature of the tendon graft is highly susceptible to the rate of loading. Theoretically, the same volume of extracellular fluid can be extruded from a graft under the same loads. However, it was noted that the graft requires time to adapt to the compressive loading and that even low loads can lead to plastic deformation and rupturing of the graft bundles. This failure of the graft was found to occur at both high (approximately, 9,000 N) and low (approximately, 1,000 N) loads, highlighting the effect that high strain rates alone can have on viscoelastic materials, such as a tendon graft. Future studies could investigate the efficacy and repeatability of higher compressive strain rates, as higher rates would result in a shorter preconditioning time, which would be desirable in any surgical setting.

Alternative procedures to decrease the water content may include the use of chemicals or heat; however in our opinion, the above-described technique appears to cause minimal damage to the tissue and anticipates physical con-

ditions to which the transplant will be subjected anyway during an implantation procedure, such as interference screw fixation. Additionally, the potentially beneficial effect of gain in length will most likely not occur through drying or chemical treatment.

## Conclusion

In summary, the volume of an 8-mm diameter and 3-cm long tendon graft may be reduced by up to 30 % by applying external pressure of 6,000 N within few minutes through extrusion of water. Simultaneously, the tendon may be pre-stretched by 10 % of its length. This finding has to our knowledge not been described before and may be considered and exploited in surgical procedures where external pressure occurs on a tendon or transplant. From a practical perspective, a compressed tendon graft may allow drilling a smaller bone tunnel, retaining more of the original bone. At the same time, the collagen content of the transplant is preserved and a tight fit of the transplant in the bone tunnel achieved.

**Conflict of interest** None.

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