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ORIGINAL PAPER

Effects of freezing on the biomechanical and structural properties of human posterior tibial tendons

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Abstract This work analyzes the effects of storage by fresh-freezing at −80°C on the histological, structural and biomechanical properties of the human posterior tibial tendon (PTT), used for ACL reconstruction. Twenty-two PTTs were harvested from eleven donors. For each donor one tendon was frozen at −80°C and thawed in physiological solution at 37°C, and the other was tested without freezing (control). Transmission electron microscopy (TEM), differential scanning calorimetry (DSC) and biomechanical analysis were performed. We found the following mean changes in frozen-thawed tendons compared to controls: TEM showed an increase in the mean diameter of collagen fibrils and in fibril non-occupation mean ratio, while the mean number of fibrils decreased; DSC showed a decrease in mean denaturation temperature and denaturation enthalpy. Biomechanical analysis showed a decrease in ultimate load and ultimate stress, an increase in stiffness and a decrease in ultimate strain of tendons. In conclusion fresh-freezing brings about significant changes in the biomechanical and structural properties of the human PTT. A high variability exists in the biophysical properties of tendons among individuals and in the effects of storage on tendons. Therefore, when choosing an allograft tendon, particular care is needed to choose a biomechanically suitable graft.

Résumé Ce travail a pour but d'analyser les effets du stockage à −80°C sur le plan histologique, structurel et biomécanique d'un tendon le tibial ou jambier postérieur (PTT), utilisé pour la reconstruction des ligaments croisés antérieurs. 22 PTT ont été conservées provenant de 11 donneurs. Pour chaque donneur un tendon a été congelé à −80°C et l'autre, conservé dans une solution physiologique à 37°C. Ces tendons ont été testés. L'examen par microscope électronique (TEM), le scanner calorimétrique (DSC) et une analyse biomécanique ont été réalisés. Nous avons trouvé des changements dans les tendons conservés au froid en comparaison du groupe contrôle. Le TEM, examen au microscope électronique a montré une diminution du diamètre des fibres collagènes. L'analyse biomécanique a montré également une diminution de la résistance à la charge et au stress ainsi qu'une augmentation de la rigidité et une diminution des contraintes terminales au niveau du tendon. En conclusion: la congélation des tendons frais amène des modifications significatives des caractéristiques biomécaniques et structurelles du tendon PTT humain. Il existe une variation importante des propriétés biophysiques des tendons parmi les individus et du fait de leurs conservations. Pour cela, il est nécessaire lorsque l'on choisit un tendon et une allogreffe du tendon d'apporter dans le choix sur le plan biomécanique un soin particulier.

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Introduction

In orthopaedic reconstruction surgery, and especially in anterior cruciate ligament reconstruction, autologous tendons are commonly used. The most frequently used are patellar tendon, semitendinosus and gracilis and quadriceps tendon. The reason for choosing these tendons is the suitability of their anatomical and biomechanical properties for elective replacement [9, 19, 27, 32].

Another suitable graft choice is represented by tendon allografts [3, 11, 12, 14, 15, 18, 21, 24, 25, 30]. The advantages of using allografts include the lack of donor-site morbidity, a reduction in surgery time and hospitalisation, and a better cosmetic result. On the other hand, disadvantages include the lack of donors, high cost, slower remodelling, risk of transmitting infectious diseases and possibility of immune reactions. The latter two problems in particular have required the implementation of preservation and sterilization techniques that have potentially negative effects on the quality of the graft.

The most commonly used preservation method is fresh-freezing at −80°C: this method effectively eliminates the risk of transplant rejection, determining cell necrosis and loss of immunogenicity [7].

However, the literature gives discordant information on the effects of freezing on the initial mechanical properties of tendons and ligaments. Most studies, carried out on animal tendons or ligaments, do not report any reduction in the main mechanical properties after freezing [2, 5, 20, 29, 33]. Other studies report various biomechanical changes related to storage [4, 26] or in situ freezing [8, 22].

With regard to the effects of freezing on the structural properties of tendons, the only available data are obtained from in situ freezing and stress-shielding on animal tendons [8, 13, 22, 28].

Many studies exist, assessing the biomechanical suitability of the various allograft tendons [9, 10, 19, 23, 27]. All these studies, however, report the average values of the main biomechanical properties of a sample of tendon grafts. In other studies the reported biomechanical values of an allogenic tendon are not much higher than those of a normal ACL [7, 31]. The variability in biomechanical data is probably related to the high variability among individuals, and so to the sample chosen for testing, as well as to the testing method.

The aim of this study is to clarify the effects of preservation by fresh-freezing, and to know whether the effects of fresh-freezing has any influence in the choice of an allograft tissue. We studied the fresh-frozen human posterior tibial tendon with regards to its use in reconstructive orthopaedic surgery, analysing comparatively any histological, structural and biomechanical changes which could be connected to the method of preservation.

Materials and methods

Twenty-two posterior tibial tendons were harvested from 11 donors aged between 25 and 59 years (mean=43.8). The entire length of the tendons was harvested (15–20 cm), from the myotendinous junction to the bone insertion. A bone block from the distal bone insertion was also harvested.

Of the 11 pairs of tendons, three pairs were used for histological analysis under light microscope (LM) and transmission electron microscope (TEM), three for calorimetric analysis, and five for biomechanical analysis. We examined the segment of the tendons between 20 to 60 mm proximal to the distal bone insertion.

For each donor, one tendon (control) was examined fresh, immediately after harvesting. The controlateral one was preserved for 30 days by fresh-freezing at −80°C and then examined after being thawed by immersion in physiological solution at 37°C for 15 minutes. We randomly assigned the right or left tendon to the control or to the frozen-thawed group.

Histological analysis

The tendon graft was divided into two segments. One was processed by LM by fixing in 10% formalin, dehydrating in ascending alcohol and embedding in paraffin. The 8 μ serial cross-sections were stained by Mallory-Azan and observed under the Leitz-Orthoplan light microscope.

The other segment was subdivided into samples for TEM observation: the samples were fixed in Karnovsky with the addition of 0.1% tannic acid, post-fixed in 1% osmium tetroxide, dehydrated in alcohol and embedded in Araldite. The fine cross-sections from the samples were then contrasted with uranyl acetate and lead citrate and examined by a Philips CM-10 electron microscope. Morphometric analysis was performed by the Leica-Quin (Germany) image analyser. Five TEM photomicrographs at a final magnification of 39,000x were randomly chosen and, successively, one single 1.5 μ^2 cross-sectional area within each photomicrograph was randomly selected. For each 1.5 μ^2 area, the Leica-Quin software provided the following data: a) the number of collagen fibrils; b) the area of each fibril; c) the perimeter of each fibril.

In order to conduct a statistical comparison between the two groups, we chose three synthesis quantities: (1) number of collagen fibrils; (2) mean diameter of collagen fibrils; (3) mean interfibrillar gap.

The *number of collagen fibrils (n)* was directly provided by the image analysis software. The *diameter* (*d*) of each single fibril, which was indispensable for calculating the fibril mean diameter. Besides, the cut plane was not perfectly perpendicular to the fibril axis, so that the

(circular) cross section of the fibrils showed an elliptical form. The minor axis of the ellipse was considered as the real diameter of the fibril. As regards the *mean interfibrillar gap (MIG)*, it was calculated as follows: $MIG=(A_{nf}/A_v)/n$, where A_{nf} denotes the area not occupied by collagen fibrils, A_v the whole visualised area and n the number of fibrils in A_v. To read *MIG* as a percentage, the ratio was multiplied by 100: its value represents the percentage of the whole visualised area which, in the examined section, surrounds, on average, a collagen fibril.

Differential scanning calorimetry

Calorimetric measurements were performed using a Perkin Elmer DSC-7, equipped with a model P_{II} intracooler. Temperature and enthalpy calibration was performed by using high purity standards (n-decane, benzene and indium). Frozen-thawed and control tendons were air dried, and then rehydrated in physiological solution at 37°C for 1 h. The measurements were carried out on known amounts of tendons (3–4 mg of dried sample). The rehydrated samples were wiped with filter paper to remove excess liquid and hermetically sealed in aluminium pans to prevent any loss of liquid during measurements.

Heating was carried out at 5°C/min in the temperature range from−5°C to+120°C. On heating in a calorimeter the collagen molecules undergo an abrupt helix to coil transition giving rise to a sharp endothermic peak at a defined temperature [16]. Denaturation temperature (T_D) was determined as the peak value of the corresponding endothermic phenomena.

The value of denaturation enthalpy (ΔH) was calculated with respect to the weight of the air-dried sample. This quantity is related to the relative amount of triple helical structure in the samples [1, 17]: thus, the values of denaturation enthalpy can be used to calculate the mean triple helix content of the frozen-thawed tendons (F), with respect to control tendons (C), as follows: $\Delta H_F/\Delta H_C*100$.

Biomechanics

Before the test, the tendons were prepared by muscle tissue debridement. Tendons were fixed by special clamps with milled plates designed by CITIEFFE (Calderara di Reno, Bologna, Italy), which had already been used for similar tests [6]. Tendon fixing was assured by freezing the clamps by putting dry ice in special containers adjacent to the clamps. This method has been reported widely in the literature [9, 10, 23, 27]. The distal bone insertion was left just outside one of the clamps (Fig. 1a).

The MTS 858 mini-bionix II was used to test the materials, having a load cell with a maximum capacity of 15 kN, provided by CITIEFFE.

A preload of 50N was maintained for 2 minutes. During this time the tendons were wet with physiological solution to keep them hydrated and avoid the free parts freezing up.

The free length of the tendons was measured by a micrometric caliper. The cross-sectional area was calculated by measuring the width and thickness in three points equidistant along the tendon with the micrometric caliper, and by calculating the mean of the three areas approximated to a rectangle [27, 31].

The tests were performed at 100% /sec. of the initial length. In all cases tendon failure occurred in the mid-substance (Fig. 1b).

Ultimate load, ultimate deformation and stiffness (calculated from the linear part of the curve) were obtained from the load/deformation curve. Ultimate stress, ultimate strain were obtained from the strain/stress curve. Young's

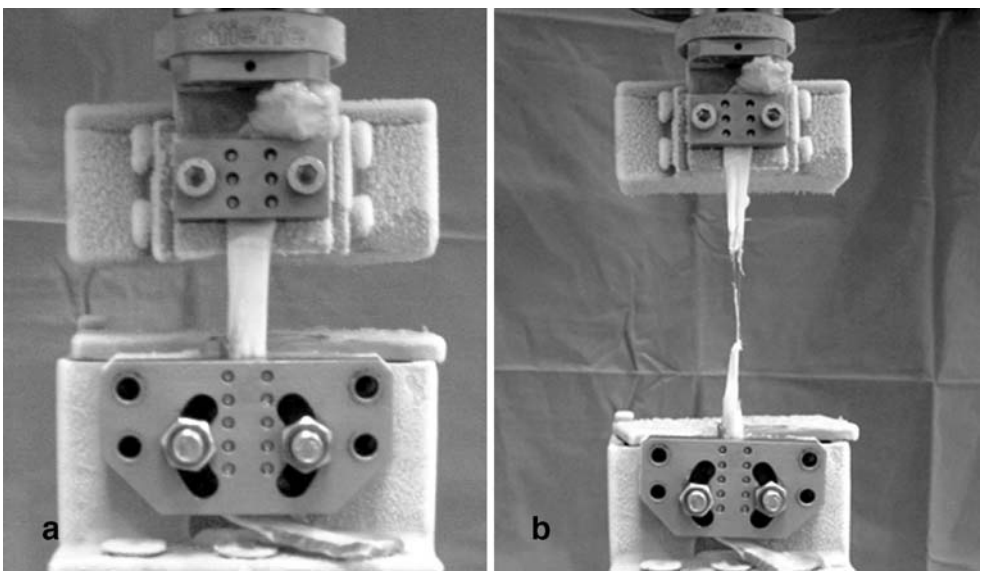

Fig. 1 Biomechanical test of tendons. (**a**) The tendon is fixed in the frozen clamps with the distal bone insertion outside the upper clamp, and under 50 N pre-loading. (**b**) The tendon undergoes mid-substance failure

modulus was calculated as follows: *stiffness* × *initial length / cross-sectional area*.

Statistical analysis

The effects of fresh-freezing and subsequent thawing on histological, structural and biomechanical properties of human PTT were investigated by analysing the statistical significance of within-subjects differences. In particular, a Wilcoxon test was used on calorimetric and biomechanical single-paired data, while a Kruskal-Wallis test was conducted on histological multiple-paired data.

Distribution-free tests (more conservative than parametric tests: they make rejection of the null hypothesis more difficult than it is by using a parametric test) were adopted owing to the non-normality and heteroscedasticity of data.

A p-value of less than 0.05 was considered to be statistically significant for all comparisons.

Results

Histological analysis

By LM (Fig. 2) the fresh tendons showed compact and lightly stained bundles of collagen fibres, running parallel to the major axis of the tendon. In the freeze-thawed samples the bundles were smaller and less tightly packed together; their course, moreover, appeared wavy.

By TEM (Fig. 3), the analysis of transverse sections showed, in fresh tendons, collagen fibrils of various diameters, and packed together. Elastic fibres were well represented. Cells showed abundant cytoplasm, rich in rough endoplasmatic reticulum (RER). In the freeze-thawed tendons the fibrils appeared to be enlarged and separated by wider interfibrillar gaps. The elastic fibres did not show any variation. The cells presented an electrodense nucleus and cytoplasm devoid of RER, which indicates cell death.

Regarding the morphometric analysis, the collagen fibril diameters ranged in control tendons from 10 to 140 nm, with the higher densities associated to intervals between 10 and 40 nm; another peak, though not so high as the previous one, corresponded to larger diameter values. The diameter histograms for the freeze-thawed tendons showed, in each subject, a wider range and a shift in the frequencies towards larger diameters, reaching to 200 nm (Fig. 4).

Table 1 shows some descriptive statistics (means and standard deviations) of the morphological parameters of interest in control and frozen-thawed tendons. Particularly,

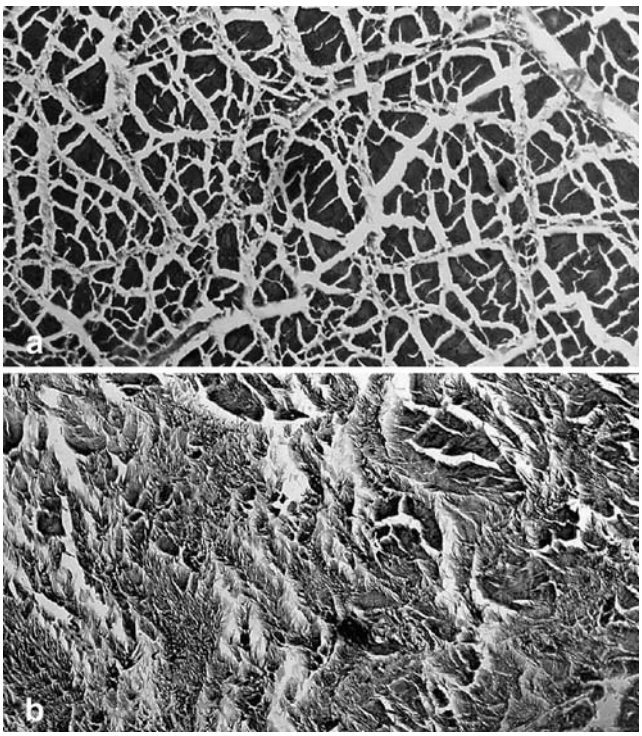

Fig. 2 Analysis of cross-sections by LM 40x. (**a**) The fresh tendon shows the collagen fibres tightly packed and grouped into bundles. (**b**) The controlateral tendon after freezing shows splitting and fragmentation of the collagen fibres, which are divided by a greater amount of ground substance

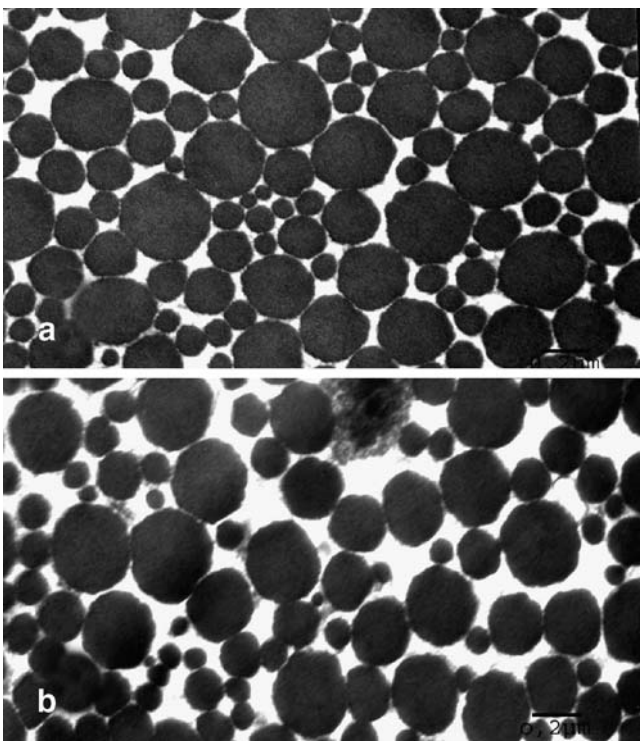

Fig. 3 Analysis of cross-sections by TEM 39,000x. (**a**) The fresh tendon shows collagen fibrils of various diameters; the percentage of smaller diameters is higher. The fibrils are closely packed together. (**b**) The controlateral frozen-thawed tendon shows collagen fibrils of various diameters; the percentage of smaller diameters is lower. The fibrils are less tightly packed and divided by wider interfibrillar gaps

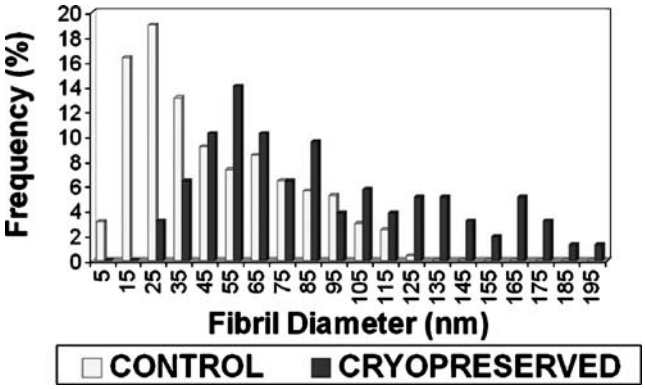

Fig. 4 Histograms of collagen fibril diameters in a control tendon and a frozen-thawed one. The fresh tendon shows fibrils with a diameter ranging between 10 and 140 nm with a peak between 10 and 40 nm and another peak for larger diameters (60–70 nm). The controlateral tendon shows fibrils with diameters of up to 200 nm, with the first peak for diameters of 40–70 nm

the mean values in Table 1 were "hierarchically" computed by averaging the fibril mean diameter, the mean interfibrillar gap and the number of fibrils-observed in each sampled 1.5 μ^2 area-first within each single donor and successively over all the three donors. Over the donor averages, standard deviations were also calculated and showed in the table.

It is worth noting from Table 1 that the fibril mean diameter in the frozen-thawed specimens (77.3±15.1 nm: MEAN±SD) was greater than it was in the control ones (47.0±8.1 nm), with a statistically significant mean increase of 30.3±16.2 nm ($p<0.0001$). A statistically significant mean increase of 1±0.7% ($p<0.0001$) was also observed in the mean interfibrillar gap (1.3±0.7% in the frozen-thawed specimens *versus* 0.3±0.1% in the control ones). As a result of both increases in fibril mean diameter and spacing among fibrils, a mean decrease of 122±47.2 units in the number of collagen fibrils (57.8±19.40 units in the frozen-thawed specimens *versus* 179.8±57.2 units in the control ones) was registered in the examined 1.5 μ^2 areas and was demonstrated statistically significant ($p<0.0001$).

Table 1 Effects of storage (histological analysis)

		Control	Frozen-thawed	Variation
Mean fibril diameter (nm)	Mean	47.0	77.3	30.3
	SD	8.1	15.1	16.2
Mean interfibrillar gap (%)	Mean	0.3	1.3	1.0
	SD	0.1	0.7	0.7
Number of fibrils (units)	Mean	179.8	57.8	−122.0
	SD	57.2	19.4	47.2

Table 2 Effects of storage (Differential Scanning Calorimetry)

		Control	Frozen-thawed	Variation
Denaturation temperature (°C)	Mean	68.3	64.7	−3.7
	SD	2.6	1.9	0.9
Denaturation enthalpy (J/g)	Mean	44.5	38.3	−6.2
	SD	1.3	2.2	1.3
Amount of triple helix (%)	Mean	100	85.9	−14.1
	SD	0.0	3.1	3.1

Differential scanning calorimetry

Results of the DSC analysis revealed, in the examined subjects, a mean decrease of 3.7±0.9°C in the denaturation temperature of the frozen-thawed tendon with respect to the control sample (Table 2), in agreement with a reduction in collagen thermal stability. The denaturation enthalpy underwent a mean decrease of 6.2±1.3 J/g. Accordingly, on average, the amount of triple helix structure of the frozen-thawed specimens, evaluated from the enthalpy values, accounted for 85.9±3.1% with respect to the control samples. However, the observed differences between frozen-thawed tendons and control tendons were not demonstrated statistically significant ($p>0.05$).

Biomechanics

The free length of tendons was 39.8 mm (SD=1.8) for fresh tendons, and 40.6 mm (SD=2.0) for frozen-thawed tendons.

Table 3 shows the average values for all the biomechanical parameters for control and frozen-thawed tendons. We

Table 3 Effects of storage (biomechanics)

		Control	Frozen-thawed	Variation
Cross-sectional area (mm^2)	Mean	21.9	25.6	3.7
	SD	2.3	2.6	1.7
Ultimate load (N)	Mean	2517.4	2060.0	−457.4
	SD	270.8	233.4	176.9
Ultimate deformation (mm)	Mean	7.9	5.3	−2.6
	SD	1.2	1.7	0.9
Stiffness (N/mm)	Mean	438.2	513.9	75.7
	SD	77.6	79.1	23.4
Ultimate stress (MPa)	Mean	117.3	81.9	−35.4
	SD	25.1	16.9	16.0
Ultimate strain (%)	Mean	19.8	13.0	−6.8
	SD	3.1	4.0	2.5
Young's modulus (MPa)	Mean	807.8	807.5	0.3
	SD	148.4	137.2	70.0

gave emphasis to the standard deviation (SD) among individuals in both the control and frozen-thawed groups, and among variations between the groups.

Fresh tendons had a mean cross-sectional area of 21.9 mm^2; in frozen-thawed tendons the mean cross-sectional area was 25.6 mm^2. Accordingly, there was an average increase of 17%.

The mean structural properties of tendons showed the following variations after storage: ultimate load had a decrease of 457.4 N (−18.2%); ultimate deformation had a decrease of 2.6 mm (−32.8%); stiffness had an increase of 75.7 N/mm (+17.3%).

The mean material properties reported the following variations: ultimate stress decreased 35.4 MPa (−30.2%); ultimate strain decreased 6.8% (−34.5%); Young's modulus did not vary greatly (−0.3 MPa).

The variations in all the parameters (except for the Young's modulus) were statistically significant ($p<0.05$).

Discussion

We chose to assess the properties of human posterior tibial tendon because it is very suitable as a replacement tissue in orthopaedic reconstruction surgery and especially in ACL reconstruction. In fact, it is a long tendon (15–20 cm), that enables a secure fixation with the help of the bony insertion at its distal end. Its flat shape allows its free end to be fixed either by an interference screw or a staple.

Histological analysis showed significant changes after storage; there was an increase in the mean diameter of fibrils, with the small fibrils being less represented. Interfibrillar gaps are also increased, resulting in a decreased number of collagen fibrils per sample. Tsuchida et al. (1997) [28] reported similar results in in situ frozen and stress-shielded rabbit patellar tendon, demonstrating a decrease in the number of fibrils and in the fibril occupation ratio at 3 and 6 weeks after freezing. They observed a decrease in small diameter fibrils and an increase in fibrils with a diameter greater than 360 nm. This swelling phenomenon, as well as the splitting and fragmentation of collagen bundles observed by LM, can probably be attributed to ice crystals formation [28].

The decrease in collagen fibril density is supported by calorimetric analysis, and in particular by the reduction in denaturation enthalpy, which means decreased triple helix content per mass unit.

The histological observations are associated with the increase of the cross-sectional area in the freeze-thawed tendons. This phenomenon has already been described in the literature [8, 13, 22].

The major debate continues over the effects of storage on the biomechanical properties of tendons and their relationship with the histological findings. Most authors do not find any relevant change in the mechanical properties when comparing fresh and frozen-thawed tendons or ligaments [2, 5, 22, 29]. We found a decrease in ultimate load, which can be explained by splitting and fragmentation of collagen bundles; from an ultrastructural point of view, the decrease in ultimate tensile stress is well explained by the decrease in collagen fibril density and in triple helix content per mass unit. It has been reported that a damaged tendon displays a reduction in denaturation enthalpy, which was interpreted as a proportion of the molecules being denaturated [17]. These results are in agreement with Clavert et al. (2001) [4], who demonstrated a decrease in ultimate tensile load when comparing fresh and frozen-thawed tendons (human long head of the biceps brachii); and with Ohno et al. (1993) [22] and Jackson et al. (1991) [13], who showed a decrease in ultimate tensile stress in in situ frozen rabbit patellar tendons. Jackson et al. (1991) [13] found that this mechanical loss appeared before the collagen remodelling had started: so he attributed this phenomenon to the direct effect of freezing.

At variance with the results of previous studies [4, 13, 22, 26], we did not find any variation in Young's modulus of frozen-thawed tendons probably because of the decrease in ultimate strain which occurs simultaneously to that in ultimate load.

There are many studies assessing the biomechanical suitability of various allograft tendons [9, 10, 19, 23]; but very different biomechanical values are reported in the literature [27, 31], depending on the sample and on the testing method, with some sample reporting values lower than those of a normal ACL [32].

If we consider: (a) the high variability in the biomechanical properties among individuals, due to differences in age, sex, body weight, work or sports activity, and in the presence of pathologies, conditions or drugs which affect the biomechanics of tendons; (b) the variability in the effects of preservation on tendons: the allogenic tendon grafts will not always fulfil the biomechanical requirements for ACL reconstruction.

In conclusion, particular care is required when choosing an allograft tendon and further studies are required to clarify the role of donors' age and sex, and other factors, in determining the suitability of an allogenic tendon. The future directions could be: (a) to choose the tendon grafts based the donors' characteristics; (b) to use fresh tendons; (c) to test the controlateral tendon from the same donor before use.

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References

1. Achet D, He XW (1995) Determination of the renaturation level gelatin films. Polymer 36:787–791
2. Barad S, Cabaud HE, Rodrigo JJ (1982) Effects of storage at−80°C as compared to 4°C on the strength of Rhesus Monkey anterior cruciate ligaments. Transactions of the Annual Meetings of the Orthopaedic Research Society 7:378
3. Caborn DNM, Selby JB (2002) Allograft anterior tibialis tendon with bioabsorbable interference screw fixation in anterior cruciate ligament reconstruction. Arthroscopy 18(1):102–105
4. Clavert P, Kempf JF, Bonnomet F et al (2001) Effects of freezing/thawing on the biomechanical properties of human tendons. Surg Radiol Anat 23:259–262
5. Dorlot JM, Ait ba Sidi M, Grembley GM, Drouin G (1980) Load-elongation behaviour of the canine anterior cruciate ligament. J Biomech Eng 102:190–193
6. Ferretti A, Conteduca F, Morelli F et al (2003) Biomechanics of ACL reconstruction using twisted doubled hamstring tendons. Int Orthop 27:22–25
7. Gitelis S, Cole BJ (2002) The use of allografts in orthopaedic surgery. Instr Course Lect 51:507–525
8. Graf BK, Fujisaki K, Vanderby R, Vailas AC (1992) The effect of in situ freezing on rabbit patellar tendon (A histologic, biochemical and biomechanical analysis). Am J Sports Med 20(4):401–405
9. Hamner DL, Brown CH, Steiner ME et al (1999) Hamstring tendon grafts for reconstruction of the anterior cruciate ligament: Biomechanical evaluation of the use of multiple strands and tensioning technique. J Bone Joint Surg 81-A:549–557
10. Haut Donahue TL, Howell SM, Hull ML, Gregerson C (2002) A biomechanical evaluation of anterior and posterior tibialis tendons as suitable single-loop anterior cruciate ligament grafts. Arthroscopy 18(6):589–597
11. Indelicato PA, Linton RC, Huegel M (1992) The results of fresh-frozen patellar tendon allografts for chronic anterior cruciate ligament deficiency of the knee. Am J Sports Med 20(2):118–121
12. Indelli PF, Dillingham MF, Fanton GS, Shurman DJ (2004) Anterior cruciate ligament reconstruction using cryopreserved allografts. Clin Orthop 420:268–275
13. Jackson DW, Grood ES, Cohn BT et al (1991) The effects of in situ freezing on the anterior cruciate ligament. J Bone Joint Surg 73-A(2):201–213
14. Kuechle DK, Pearson SE, Beach WR et al (2002) Allograft anterior cruciate ligament reconstruction in patients over 40 years of age. Arthroscopy 18(8):845–853
15. Linn RM, Fischer DA, Smith JP et al (1993) Achilles tendon allograft reconstruction of the anterior cruciate ligament-deficient knee. Am J Sports Med 21(6):825–831
16. Miles CA, Bailey AJ (1999) Thermal denaturation of collagen revisited. Proc Indian Acad Sci Chem Sci 111:71–80
17. Miles CA, Wardale RJ, Birch HL, Bailey AJ (1994) Differential scanning calorimetric studies of superficial digital flexor tendon degeneration in the horse. Equine Vet J 26:291–296
18. Noyes FR, Barber SD, Mangine RE (1990) Bone-Patellar ligament-Bone and fascia lata allografts for reconstruction of the anterior cruciate ligament. J Bone Joint Surg 72-A(8):1125–1136
19. Noyes FR, Butler DL, Grood ES et al (1984) Biomechanical analysis of human ligament grafts used in knee-ligament repairs and reconstructions. J Bone Joint Surg 66–A:344–352
20. Noyes FR, Grood ES (1976) The strength of the anterior cruciate ligament in human and Rhesus monkeys. Age-related and species-related changes. J Bone Joint Surg 58-A:1074–1082
21. Nyland J, Caborn DNM, Rothbauer J et al (2003) Two-year outcomes following ACL reconstruction with alograft tibialis anterior tendons: a retrospective study. Knee Surg Sports Traumatol Arthrosc 11:212–218
22. Ohno K, Yasuda K, Yamamoto M et al (1993) Effects of complete stress-shielding on the mechanical properties and histology of in situ frozen patellar tendon. J Orthop Res 11:592–602
23. Pearsall AW, Hollis JM, Russell GV, Scheer Z (2003) A biomechanical comparison of three lower extremity tendons for ligamentous reconstruction about the knee. Arthroscopy 19(10):1091–1096
24. Shino K, Inoue M, Horibe S et al (1990) Reconstruction of the anterior cruciate ligament using allogenic tendon (Long-term followup). Am J Sports Med 18(5):457–465
25. Siebold R, Buelow JU, Bos L, Ellermann A (2003) Primary ACL reconstruction with fresh-frozen patellar versus Achilles tendon allograft. Arch Orthop Trauma Surg 123:180–185
26. Smith CW, Young IS, Kearney JN (1996) Mechanical properties of tendons: Changes with sterilization and preservation. J Biomech Eng 118:56–61
27. Staubli HU, Schatzmann L, Brunner P et al (1999) Mechanical tensile properties of the quadriceps tendon and patellar ligament in young adults. Am J Sports Med 27(1):27–34
28. Tsuchida T, Yasuda K, Kaneda K et al (1997) Effects of in situ freezing and stress-shielding on the ultrastructure of rabbit patellar tendon. J Orthop Res 15:904–910
29. Viidik A, Lewin T (1966) Changes in tensile strength characteristics and histology of rabbit ligaments induced by different modes of post-mortem storage. Acta Orthop Scand 37:141–155
30. Wainer RA, Clarke TJ, Poehling GG (1988) Arthroscopic reconstruction of the anterior cruciate ligament using allograft tendon. Arthroscopy 4(3):199–205
31. Wilson TW, Zafuta MP, Zobitz M (1999) A biomechanical analysis of matched bone-patellar tendon-bone and doubled-looped semitendinosus and gracilis tendon grafts. Am J Sports Med 27(2):202–207
32. Woo SL, Hollis JM, Adams DJ et al (1991) Tensile properties of the human femur-anterior cruciate ligament-tibia complex (The effects of specimen age and orientation). Am J Sports Med 19(3):217–225
33. Woo SL, Orlando CA, Camp JF, Akeson WH (1986) Effects of post-mortem storage by freezing on ligament tensile behaviour. J Biomech 19(5):399–404