Assessment of human patellar tendon composition in situ using near-infrared spectroscopy

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Disclosures: Heikki Kröger (9; Nordic Orthopaedic Federation).

INTRODUCTION: Current methods for directly assessing human tendon properties *in vivo* are either not clinically feasible (e.g., buckle transducers and needle-inserted optic fibers), are limited to a minute region of the tendon (e.g., biopsies), or require complex setups involving multiple measurement systems (e.g., a combination of motion capture, ultrasonography, and isokinetic dynamometry). As such, tendon pathologies are notoriously challenging to diagnose, monitor, and rehabilitate, and thus new methodologies for fast and accurate predictions of human tendon properties are warranted. Near-infrared (NIR) spectroscopy (NIRS) is a vibrational spectroscopic technique shown to provide a rapid, non-destructive assessment of human bone, cartilage, and meniscus properties [1]. NIRS previously demonstrated great potential in estimating the composition and mechanical properties of bovine knee ligaments and patellar tendon [2,3]. However, NIR spectra in those studies were acquired from prepared samples and not from whole intact ligaments or tendons [2,3]. Further, the methodology should also be verified with intact human tissue. Given that the composition of a tendon influences its function [4] and tendon composition is altered in a diseased state [5], in turn also impairing its function [6], the ability to predict human tendon composition in a clinically translatable setting would position NIRS as a valuable tool for assessing tendon function and health. Thus, the aim of this study was to investigate whether NIR spectra obtained *in situ* from intact cadaveric patellar tendon can be used to estimate important compositional constituents of the tissue.

METHODS: The Research Ethics Committee of the Northern Savo Hospital District gave a favourable opinion towards the study protocol (134/2015). NIR spectra were obtained from medial, central, and lateral patellar tendon regions of eight fresh-frozen cadaver knees (five female; 65 ± 8 yr; 1.72 ± 0.15 m; 83 ± 23 kg) using a Stellarnet spectrometer (DS-InGaAs-512). Knees were placed in ~90° of knee flexion, and NIR spectra were obtained directly from patellar tendons *in situ* via small incisions through the skin (Figure 1). Subsequently, each patellar tendon was harvested and prepared into six regional pieces (three mediolateral × two anteroposterior). Each region was subjected to biomechanical (results presented elsewhere [7,8]) and biochemical tests. After mechanical testing to failure, the broken ends of the testing piece were frozen at -80°C for later analyses of water, hydroxyproline (collagen), and uronic acid (proteoglycan) contents, the latter two normalized to both wet and dry weights of the sample [4]. Individual NIRS prediction models were developed for each compositional variable and evaluated using leave-one-group-out cross-validation. Regression was implemented using partial least squares [1,2,3]. As no systematic region-specific differences in patellar tendon material properties [7] or composition (region-specific data not presented here) were found in this dataset, data from each mediolateral region's anterior testing piece were used for the reference biomechanical data.

RESULTS: Parameter reference values and NIRS predictions are summarized in Table 1. The constructed models from NIR spectra were able to predict the water and collagen (hydroxyproline) contents of human patellar tendon with good correlation and reasonable error (i.e., $r_{CV} = 0.69$-0.74; RMSE$_{CV} = 13$-22%). Proteoglycan (uronic acid) content could only be moderately predicted (i.e., $r_{CV} = 0.36$-0.44; RMSE$_{CV} = 21$-25%).

DISCUSSION: Our aim in this proof-of-concept study was to investigate the feasibility of NIRS for predicting human patellar tendon properties. Consistent with previous results on prepared bovine knee ligament and patellar tendon samples [3], predictions from NIR spectra of the human patellar tendon obtained *in situ* were stronger for water and collagen (hydroxyproline) contents than for proteoglycan (uronic acid) content. It is likely that proteoglycan content could not be well predicted due to its relatively small amount in human patellar tendon. However, the inability of NIRS to predict uronic acid content may not necessarily be a major weakness. Rather, tendinopathic patellar tendons exhibit increased water content, with some also demonstrating greater collagen content [5]. The ~33% increase in water content shown previously in tendinopathic patellar tendons [5] well exceeds the 13% typical prediction error observed with NIRS. Additionally, lower patellar tendon viscosity is associated with worse functional performance in individuals with patellar tendinopathy [9]. As predictions of human patellar tendon viscoelastic properties were also strong with this workflow [8], the ability of NIRS to predict compositional (water and collagen) and mechanical (viscoelastic) characteristics of human tendons holds strong clinical value.

SIGNIFICANCE/CLINICAL RELEVANCE: Quantifying tendon properties with the current methodology may be useful for surgeons (e.g., prior to harvest for anterior cruciate ligament reconstruction). Further development of the NIRS probe and models of light-tendon and light-skin interactions may lead to the development of NIRS as a point-of-care tool for non-invasive and non-destructive *in vivo* characterization of human tendon properties in a clinical setting.

REFERENCES: [1] Afara IO et al. (2021) *Nat. Protoc.* 16, 1297–1329), [2] Torniainen J et al. (2019) *Ann. Biomed. Eng.* 47, 213–222, [3] Torniainen J et al. (2022) *PLoS One* 17, e0263280, [4] Ristaniemi A et al. (2020) *J. Mech. Behav. Biomed. Mater.* 104, 103639, [5] Samiric T et al. (2009) *Matrix Biol.* 28, 230–236, [6] Williamson PM et al. (2021) *Acta Biomater.* 134, 43-56. [7] Kositsky A et al. (2022) *9th World Congress of Biomechanics* O-10078, [8] Kositsky A et al. (2023) *XXIX Congress of International Society of Biomechanics* O28-4, [9] Sprague AL et al. (2022) *J. Orthop. Res.* 2320–2329.
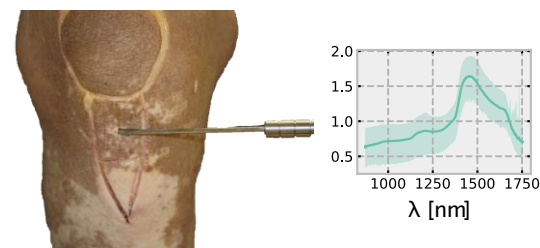
Figure 1. *Left*: Example of *in situ* acquisition of near-infrared spectra from human patellar tendon via small incisions in the skin. *Right*: Average near-infrared absorbance spectra (solid line) and 95% confidence intervals (shaded area).

Parameter	Mean	r_{CV}	RMSE$_{CV}$
Water content	$77.1 \pm 7.6\%$	0.732	12.8%
Hydroxyproline (wet weight)	23.9 ± 5.1 µg/mg	0.745	21.9%
Hydroxyproline (dry weight)	109.2 ± 18.3 µg/mg	0.692	13.1%
Uronic acid (wet weight)	0.22 ± 0.10 µg/mg	0.441	20.7%
Uronic acid (dry weight)	0.97 ± 0.34 µg/mg	0.359	25.3%

Table 1. Mean ± standard deviation of each parameter for all 48 samples subjected to testing. Calibrated (r_{CV}) and cross-validated (RMSE$_{CV}$) comparisons between measured and predicted human patellar tendon compositional properties.