Acta of Bioengineering and Biomechanics
Vol. 16, No. 3, 2014

Original paper
DOI: 10.5277/abb140302

Loading rate effect on mechanical properties of cervical spine ligaments

ANA TRAJKOVSKI*, SENAD OMEROVIC, SIMON KRASNA, IVAN PREBIL

The Chair of Modelling in Engineering Sciences and Medicine, Faculty of Mechanical Engineering, University of Ljubljana, Ljubljana, Slovenia.

Mechanical properties of cervical spine ligaments are of great importance for an accurate finite element model when analyzing the injury mechanism. However, there is still little experimental data in literature regarding fresh human cervical spine ligaments under physiological conditions. The focus of the present study is placed on three cervical spine ligaments that stabilize the spine and protect the spinal cord: the anterior longitudinal ligament, the posterior longitudinal ligament and the ligamentum flavum. The ligaments were tested within 24–48 hours after death, under two different loading rates. An increase trend in failure load, failure stress, stiffness and modulus was observed, but proved not to be significant for all ligament types. The loading rate had the highest impact on failure forces for all three ligaments (a 39.1% average increase was found). The observed increase trend, compared to the existing increase trends reported in literature, indicates the importance of carefully applying the existing experimental data, especially when creating scaling factors. A better understanding of the loading rate effect on ligaments properties would enable better case-specific human modelling.

Key words: *biomechanics, cervical spine, failure, ligaments, loading rate*

1. Introduction

Cervical spine ligaments play an important role in the spinal column stability and protection of the spinal cord [1]. The anatomical position of ligaments, bone attachment and fibrous structure define the predominant load carrying direction of ligaments, thereby enabling function of each ligament type. The ligaments limit the neck movement within the safe range. When ligaments are injured, the vertebral column or intervertebral discs have to carry additional load, and are consequently also compromised.

These ligaments are likely to be injured during vehicle collisions, both rear-end and frontal impacts [2]–[6]. Even low-speed collisions [7], [8] can cause deformations over soft tissue injury threshold, especially rear-end impacts [9], leaving the occupant with long-term or even life-threatening trauma. Thus the mechanical properties of cervical spine ligaments are of great importance for an accurate finite element model of a vehicle occupant when analyzing the injury mechanism [10]. Knowledge of mechanical properties under different loading rates, in particular, enables more precise analysis in different injury scenarios.

Human cervical spine ligaments have a non-linear strain rate, temperature and an age-dependent response [11]–[14]. In literature, there are several studies regarding mechanical properties of the three main cervical spine ligaments: the anterior longitudinal ligament (ALL), the posterior longitudinal ligament (PLL) and the ligamentum flavum (LF). The first was the study by Chazal et al. [15], in which the response of ALL and PLL at 1 mm/min was evaluated. In a detailed study by Myklebust et al. [16], the properties of ALL, PLL, LF, capsular ligament (CL) and interspinous ligament (ISL) were reported at 10 mm/s. Przybylski et al. [17] compared the response of ALL and

* Corresponding author: Ana Trajkovski, The Chair of Modelling in Engineering Sciences and Medicine, Faculty of Mechanical Engineering, University of Ljubljana, Slovenia, Aškerčeva cesta 6, 1000 Ljubljana, Slovenia. Tel: +386 1 4771 509, Fax: +386 1 4771 178, e-mail: ana.trajkovski@fs.uni-lj.si

Received: February 28th, 2013
Accepted for publication: November 22nd, 2013

PLL at a constant loading rate of 0.33 mm/s. The first comparison of static and dynamic properties of human ALL and LF, at strain rates from 2.5–2500 mm/s, was presented in the study by Butler et al. [18]. A detailed study by Yoganandan [19] reported geometric and mechanical properties of 5 cervical spine ligaments in quasi-static regime. In the study by Ivancic et al. [20], the dynamic mechanical properties of human ALL, PLL, LF, CL, interspinous and supraspinous ligaments (ISL+SSL) and middle-third disc (MTD) were determined under average peak loading rate of 723 mm/s.

Although temperature dependent response of cervical ligaments was reported [12], only few studies have been made under controlled physiological conditions. Failure properties of cervical spine ligaments (ALL, PLL and LF) under the average deformation rate of 627±203 mm/s were determined in an environmental chamber in the study by Bass et al. [11]. Under controlled physiological temperature and humidity, viscoelastic properties of ALL, PLL and LF were determined in the studies by Lucas et al. [21] and Troyer and Puttlitz [22]. Only recently in the study by Mattucci et al. [23], strain rate dependent properties of younger human cervical spine ligaments (ALL, PLL, LF, CL and ISL) have been discussed.

In literature, there are only recommendations for the soft tissue testing procedure [24]. Still, there are several documented differences in the experimental procedure that affect tissue response, such as tissue (re)freezing [25], preconditioning [26], [27] and donors' age [13], [28]. Different environmental conditions coupled with the above mentioned parameters complicate comparison of the existing studies, especially when comparing results of different studies for creating the age, gender and loading rate scaling factor.

The focus of the present study is placed on three cervical spine ligaments: ALL, PLL and LF; and a better understanding of the loading rate effect on ligaments properties, which would enable better case-specific human modelling.

2. Materials and methods

With the prior permission of the National Ethical Commission, from 13 human cervical spines (average age 68.06 ± 7.64 years, range: 59 to 84 years) a total of 13 cervical ALL, 17 PLL and 17 LF were isolated within the 24–48 hours after donors' death. Spinal levels from C2 to C7 were available. Samples were obtained and mechanical tests performed at the Faculty of Medicine, Institute of Anatomy research centre. All samples with the pre-existing bone or ligament pathology were eliminated. Anthropometric characteristics coupled with donors' age, gender, time of death, autopsy and isolation were recorded.

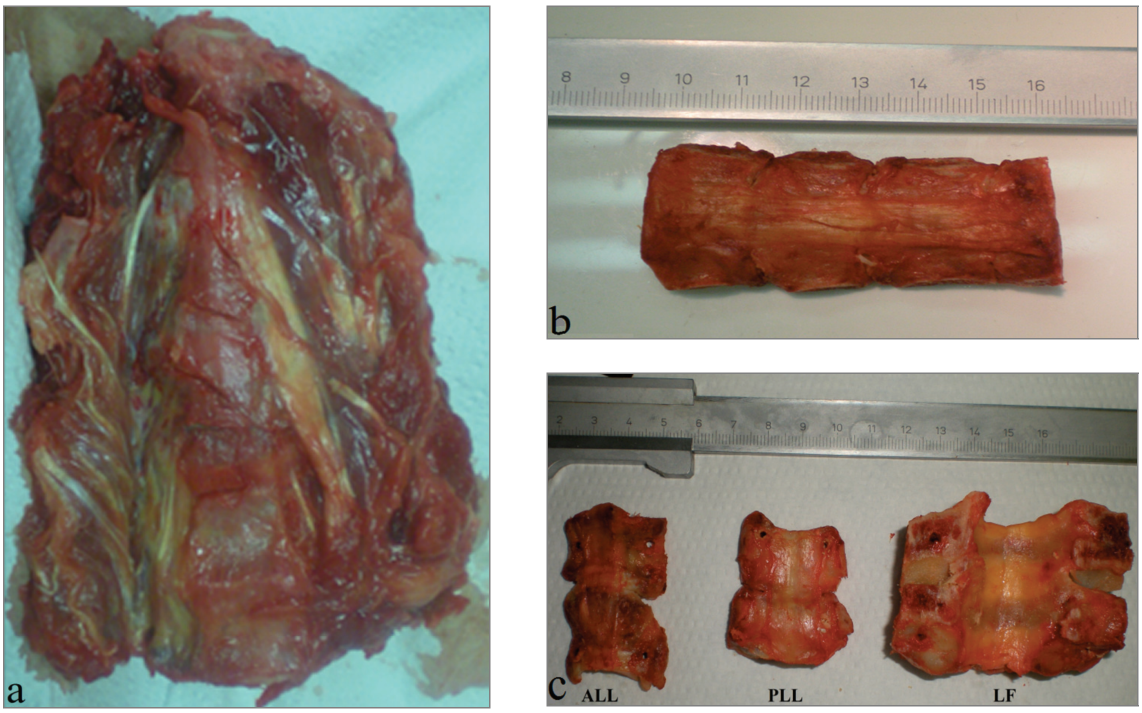

Fig. 1. Isolated cervical spine (a); PLL column (b); isolated bone-ligament-bone samples (c)

2.1. Specimen preparation

On an isolated cervical spine (Fig. 1a), vertebral bodies were separated from the vertebral arches and spinous processes. Then by sawing through the middle coronal plate of vertebral bodies, the ALL and PLL columns were divided (Fig. 1b). On the separated back part of the vertebrae, the LF column was easily observed. After inspection of ligament pre-damage, bone-ligament-bone units on a desired vertebrae were obtained (Fig. 1c) by cutting through adjacent intervertebral discs. All surrounding non-osteoligamentous tissue and intervertebral discs were carefully removed using a scalpel, anatomical tweezers and a magnifier. The LF samples were distinguished easily because of a relatively large size and a characteristic yellow colour.

After isolation, dimensions of ligaments were determined with digital callipers. The measured width and thickness in the middle of a ligament were used as ellipse axes [26], [29], when calculating the cross-sectional area. Magnifier was used when placing calliper, and special care taken not to squeeze the samples. For LF dimensions were determined on left and right arc separately, and calculated assumed elliptical areas summarized. The distance between the adjusted vertebrae was used as ligament length [11], [22], based on ligament attachment [30]. Through the isolation and measurement process, tissue hydratation was enabled with saline spray. Afterwards, prepared bone-ligament samples were wrapped in saline soaked gauze through the potting process.

Bony parts of each sample were potted into a two-component polyurethane casting resin (Fig. 2a) to enable sample attachment to a mechanical testing frame. The central position of a sample was enabled by special marks on the silicon mould and horizontal position by using medical retractors. Better attachment of bony parts was enabled with small pins placed in holes drilled through the bone (Fig. 2b).

2.2. Experimental setup

A custom designed mechanical test frame (Fig. 3) was used for a uniaxial tension test of tissue samples. The prescribed motion was enabled by a stepper motor (VRDM 3913, Berger Lahr) controlled with a stepper drive (SD326 Berger Lahr) on a linear compact module (CKK 20-145 Bosch Rexroth). Superior cross head movement was measured with a precision magnetic encoder (Elgo Electric EMIX3, with resolution 0.001 mm). Forces were measured with load cell (AEP CTS6350KC25, with 500N full scale) placed on the bottom plate. Data acquisition and control was enabled by NI DAQ card (NI USB 6221), NI Motion controller (NI PCI 7324) and LabVIEW software.

The tests were performed in an environmental chamber under physiological air temperature of 36.7 °C and air humidity of 95%. Desired conditions were kept with heated water and the air bubbler placed on the bottom of the chamber, and controlled by a thermo-hygrometer placed near the test sample.

After a 5 N tensile preload, at slow displacement rate of 0.05 mm/s, all samples were left to fully relax for 1000 s, and initial ligaments length was determined. After preload, all samples were preconditioned

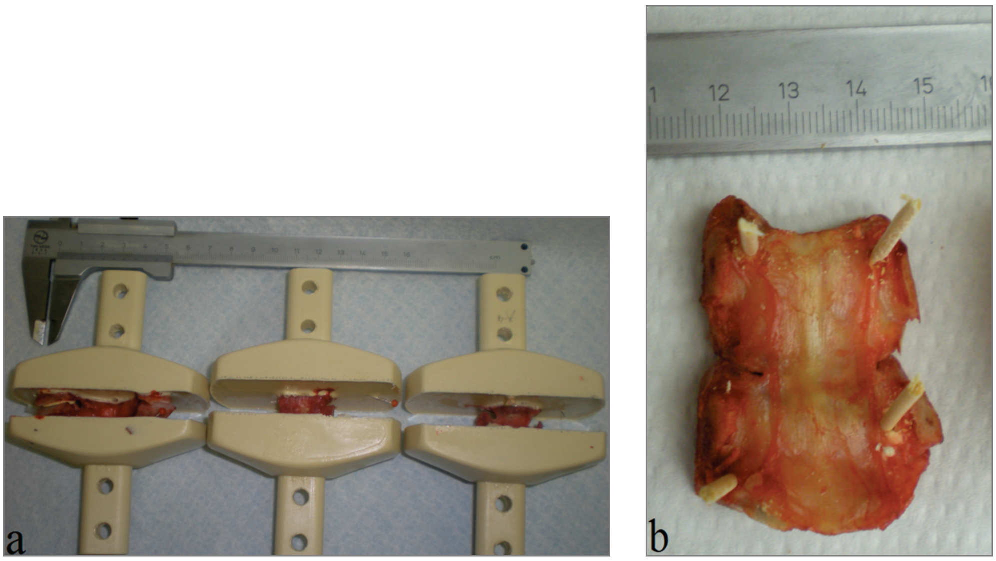

Fig. 2. Potted samples (a); better attachment of PLL with pins (b)

with 10% strain for 20 cycles at 1 Hz, and left to recover for another 1000 s [26]. All samples were loaded with constant loading rates of 2 mm/s or 15 mm/s until total rupture. These loading rates are also expected in low-speed collisions [8], [10]. Non-linear force-elongation curves were plotted and failure load, failure elongation, and linear stiffness were statistically compared among samples. Since no significant difference in the failure force or elongation among spinal levels was previously observed in literature [11], [20], and due to a limited number of available tissue samples, mean values were calculated along the cervical spine for different adjacent vertebras in this study.

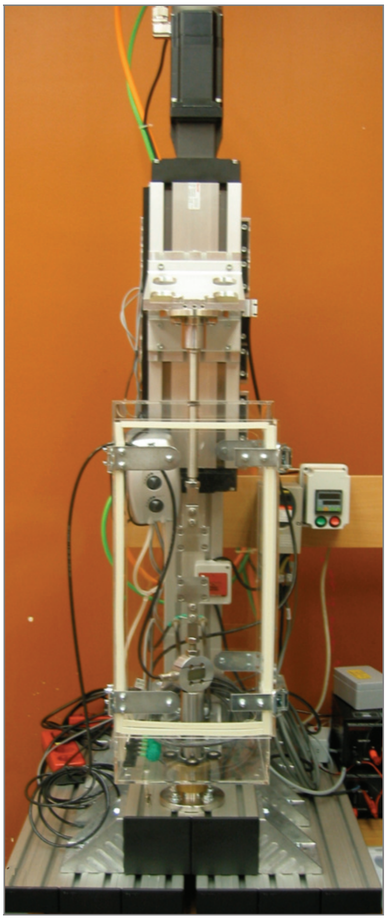

Fig. 3. Mechanical test frame

The force (elongation) after which further increase in elongation did not produce further force increase was defined as failure load (failure elongation). Stiffness was calculated as slope of the line fitted to the linear part of the load-elongation curve, between the toe and damage region (Fig. 4). The transaction point of polynomial fit to the lower portion load-elongation curve (the toe region) and linear portion of the load-elongation curve was defined as toe elongation [23]. Based on structural ligaments properties (failure load, failure elongation), in combination with geometric properties, stress-strain curves were formed, and material properties for all ligaments calculated.

The differences among the three cervical spine ligaments were analysed using analysis of variance (ANOVA) with post-hoc Tukey test for multiple comparisons. The loading rate effect on each of the properties: failure load, failure elongation, stiffness, failure stress, failure strain, modulus, toe elongation and strain, for each of the ligaments was quantified using two sample t-test. Statistical significance was set at a level 0.05.

3. Results

Measured mean initial length and cross sectional areas of all three cervical spine ligaments are represented in Table 1. There was no statistical difference in initial geometric properties between samples used for tests at two different loading rates. For both longitudinal ligaments (ALL and PLL) there was no significant difference in initial length or initial cross sectional area. LF, composed of two arches, has statistically greater cross sectional area compared to both longitudinal ligaments [1], [19], [21].

Table 1. Mean geometric properties of ligaments ± SD

Ligament type	Loading rate [mm/s]	Initial length l_0 [mm]	Initial area A_0 [mm^2]
ALL	2	3.54 ± 0.32	10.45 ± 0.59
	15	3.8 ± 0.87	10.37 ± 1.01
PLL	2	3.51 ± 0.33	13.33 ± 1.01
	15	3.54 ± 0.73	13.63 ± 1.03
LF	2	4 ± 0.73	33.59 ±6.71
	15	4.27 ± 0.66	34.17 ± 5.78

As response to uniaxial tensile load, a characteristic 4-zone (toe, linear elastic, damage, post-failure region; Fig. 4) sigmoidal load-elongation curve [19] was observed (for ALL in Fig. 5), for all ligament types and loading rates. For ALL linear part of the load-elongation curve was identified from mean value (standard deviation) of 30.37(3.9)% to 86.33(8.12)% of failure load at both loading rates. Similarly for PLL, from 30.87 (7.17)% to 81.52 (4.49)% of failure load, and from 32.52 (6.4)% to 82.46 (9.89)% of failure load for LF.

From multiple comparisons there was no statistical difference between ALL and PLL for any measured property. The difference was found between LF and both the ALL and PLL in failure force, failure stress and stiffness at both loading rates, and toe strain at 2 mm/s.

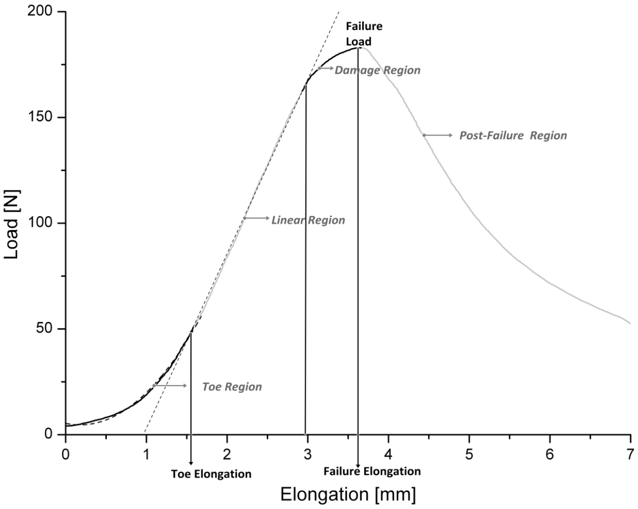

Fig. 4. Characteristic regions of load-elongation curve

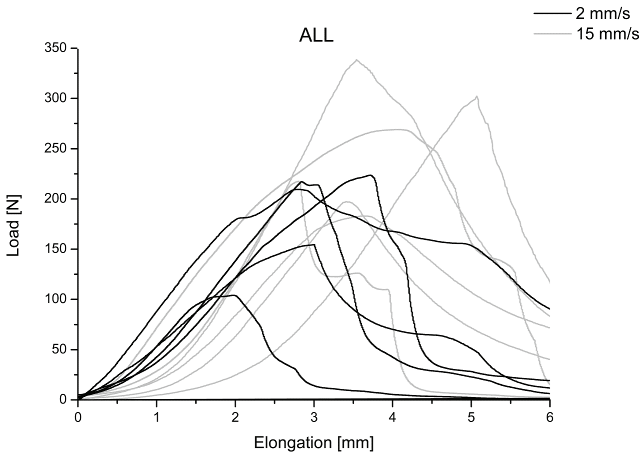

Fig. 5. Response of ALL during failure test at two loading rates

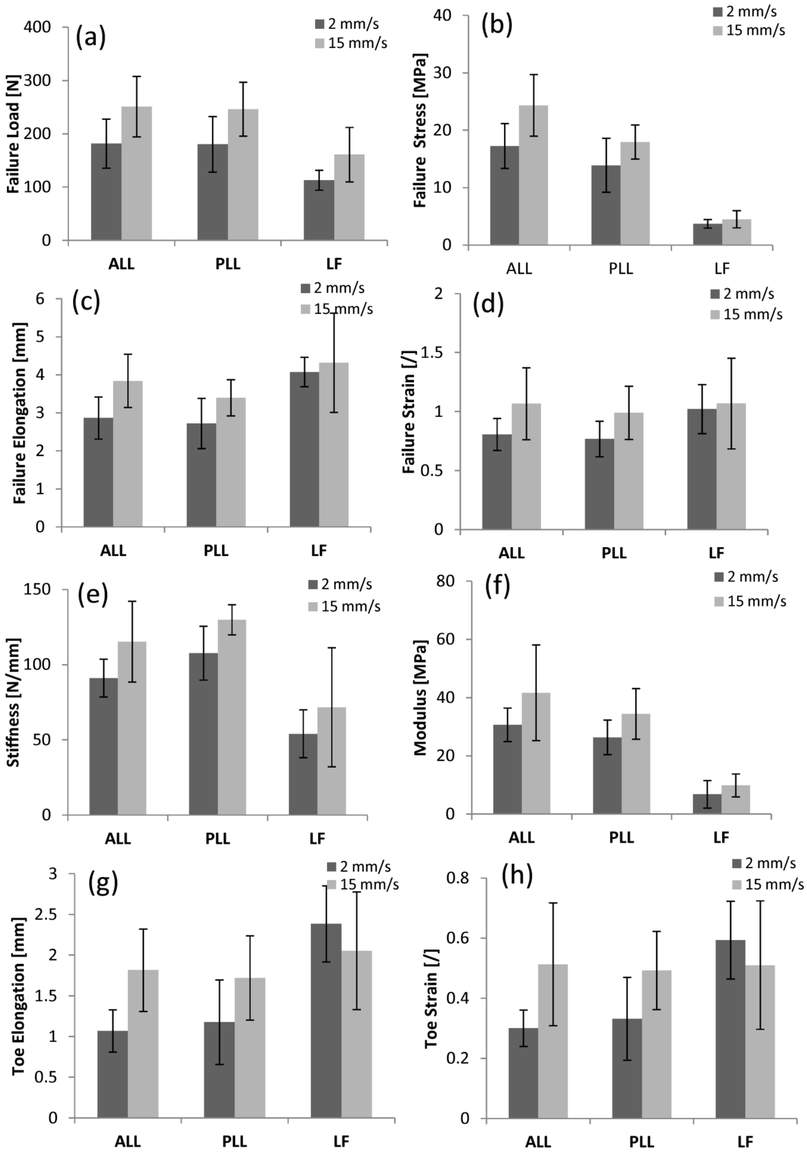

Fig. 6. The mean and standard deviation for: (a) failure load, (b) failure stress, (c) failure elongation, (d) failure strain, (e) stiffness, (f) modulus, (g) toe elongation, (h) toe strain

The mean failure load, failure elongation and stiffness, as well as calculated mean failure stress, failure strain and modulus for all ligament types at both loading rates are represented in Fig. 6a–h. Together with the increase of the loading rate, the increase trend in failure properties, stiffness and modulus was observed. The loading rate had the highest impact on failure forces for all three ligaments, a 39.1% average increase was found. For LF, failure force statistically significantly increased ($p < 0.05$), but other parameters did not significantly change ($p > 0.05$), although an increase trend in failure stress, stiffness and modulus was observed. For both longitudinal ligaments (ALL and PLL) all measured parameters increased significantly, except failure strain (ALL: $p = 0.12$; PLL: $p = 0.06$). For the ALL, the failure load increased by 38.2% ($p < 0.05$), the value of failure stress increased by 41.34% ($p < 0.05$), and modulus by 37.56% ($p < 0.05$). Similarly, for the PLL, failure load showed a 36.54% increase ($p < 0.05$), failure stress showed a 27.7% increase ($p < 0.05$), and modulus increased by 29.7% ($p < 0.05$).

4. Discussion

In the present study, mechanical properties of three fresh human cervical spine ligaments (isolated and tested within 24–48 h after donors' death) were determined under two different loading rates in physiological conditions. As expected due to their similar structure, position and function there was no statistical difference between measured properties of ALL and PLL at both loading rates, similarly as reported earlier for failure true stress and strain in study of Bass [11], and modulus, stiffness and energy to fail in srudy of Przybylski [17]. Although there was no statistical difference between ALL and PLL, PLL on average showed higher stiffness, at both loading rates. As the ligament with the highest elastin percentage, LF fails at lower failure force, failure stress and has lowest modulus as compared to both longitudinal ligaments. Similar difference was observed in study of Bass [11] at fast strain-rates.

The mean failure load and linear stiffness increased with the loading rate, but the change is not significant for all ligament types. Despite relatively small loading rate increase, in both longitudinal ligaments (ALL and PLL), significant increase of failure load, failure stress, linear stiffness and modulus was observed. LF as the ligament with highest elastin-to-collagen ratio, did not show any significant rate effects except in failure load, although an increase trend in stiffness is also present.

Since test environmental properties (both humidity and temperature) affect ligament mechanical properties [12], it is most appropriate to compare our study results with the results in similar physiological conditions. Compared to the dynamic failure forces and stresses (at 627 mm/s) in the study by Bass [11], failure forces measured at 2 mm/s in this study are 2 times smaller for LF, to 2.41 times smaller for PLL; failures stresses are 1.49 times smaller for LF to 2.37 times smaller for PLL. This dynamic to quasi-static failure force and stress ratio is in good agreement with the recently reported ratio in younger cervical spines [23] under physiological conditions. But this ratio is not in good agreement with the dynamic scaling factor reported in [8], [11], where scaling was based on experiments at room temperature. Up to now, two studies have been performed that analyse the loading rate effect on isolated bone-ligament-bone samples: the study by Butler [18], where LF and ALL were analyzed from 2.5–2500 mm/s but at room temperature, and recently the study by Matucci [23], where 5 cervical spine ligaments (among them ALL, PLL and LF) of younger spines were tested at various loading rates under physiological conditions. All other studies under various loading rates, quasi-static [16], [17], [31] and dynamic [11], [20], [21], although providing excellent background data on cervical spine ligament, are performed under different conditions, and therefore it is very hard to compare the results, as misleading estimations could be made. We believe that our study, although in a relatively small loading rate range, could be compared with the recently performed experiments under physiological conditions [11], [22], [23], giving a better insight into the loading rate effect.

The limitation of this study is related to reporting ligament material properties, since the contact method was used for cross sectional area determination, consequently influencing failure stress and modulus. A high resolution non-contact method would be better for determining ligament dimensions. Ideally, dimensions together with the dynamic cross-sectional area could be determined using the laser-scanner method [32] or ultrasound probe. But these methods should be specially adjusted for measuring small, wet samples in an environmental chamber. Another problem is precise distinguishing of ligament edges, from the surrounding soft tissue. Although we used a rough method, our measurements are in fairly good agreement with the previously reported ligament length [21], [22] and ligament cross-sectional area [19]. Another limitation is the number of tested samples, so further measurements are needed for analysing gender or ligament position effect coupled with the loading rate and age effect. For this study, based on the previous results [11], [20], properties were averaged for different cervical spine segments.

Based on the study by Panzer [8], a frontal 8–22 g impact increased the predicted strain rates in all three ligaments (from 1.4–12.85 s^{-1} in PLL, from 2.85–12.3 s^{-1} in ALL and 5.7–50 s^{-1} in LF), and a similar trend was reported for rear-end 4–10 g impacts (from 0.5–2 s^{-1} in PLL, from 1–9.5 s^{-1} in ALL and 2–12 s^{-1} in LF). Since this study indicated that even a relatively small loading rate change results in an increase of failure load and stiffness, it is of great importance to incorporate a valid dynamic scaling factor, especially in precise human modelling under different loading conditions.

Acknowledgements

We would like to thank Dr. Marija Hribernik and the technical staff from the Institute of Anatomy of the Medical faculty, at the University of Ljubljana, and Prof. Dr. Jože Balažic from the Institute of Forensics of the Medical faculty, at the University of Ljubljana, for their help during the experimental tests.

References

[1] WHITE A.A., PANJABI M.M., *Clinical biomechanics of the spine*, 2nd ed., Philadelphia, J.B. Lippincott, 1990.

[2] IVANCIC P.C., PEARSON A.M., PANJABI M.M., ITO S., *Injury of the anterior longitudinal ligament during whiplash simulation*, Eur. Spine. J., 2004, 13, 61–8.

[3] PANJABI M.M., CHOLEWICKI J., NIBU K., GRAUER J.N., BABAT L.B., DVORAK J., *Mechanism of whiplash injury*, Clin. Biomech. (Bristol, Avon), 1998, 13, 239–49.

[4] PANJABI M.M., CRISCO J.J., VASAVADA A., ODA T., CHOLEWICKI J., NIBU K., SHIN E., *Mechanical properties of the human cervical spine as shown by three-dimensional load-displacement curves*, Spine (Phila Pa 1976), 2001, 26, 2692–700.

[5] TOMINAGA Y., NDU A.B., COE M.P., VALENSON A.J., IVANCIC P.C., ITO S., RUBIN W., PANJABI M.M., *Neck ligament strength is decreased following whiplash trauma*, BMC Musculoskelet. Disord., 2006, 7, 103.

[6] PEARSON A.M., PANJABI M.M., IVANCIC P.C., ITO S., CUNNINGHAM B.W., RUBIN W., GIMENEZ S.E., *Frontal impact causes ligamentous cervical spine injury*, Spine (Phila Pa 1976), 2005, 30, 1852–8.

[7] CAPPON H. vRM, WISMANS J., HELL W., LANG D., SVENSSON M., *Whiplash injuries, not only a problem in rear-end impact*, 18th Int. Tech. Conf. Enhan. Saf. Veh., Nagoya, Japan: NHTSA, 2003.

[8] PANZER M.B., FICE J.B., CRONIN D.S., *Cervical spine response in frontal crash*, Med. Eng. Phys., 2011, 33, 1147–59.

[9] ITO S., IVANCIC P.C., PANJABI M.M., CUNNINGHAM B.W., *Soft tissue injury threshold during simulated whiplash: a biomechanical investigation*, Spine (Phila Pa 1976), 2004, 29, 979–87.

[10] OMEROVIC S., STOJANOVIĆ A., KRAŠNA S., PREBIL I., *Finite element model of human head, neck and torso for adult and 3yo child*, J. Biomech., 2012, 45(Supp 1), S205.

[11] BASS C.R., LUCAS S.R., SALZAR R.S., OYEN M.L., PLANCHAK C., SHENDER B.S., PASKOFF G., *Failure properties of cervical spinal ligaments under fast strain rate deformations*, Spine (Phila Pa 1976), 2007, 32, E7–13.

[12] BASS C.R., PLANCHAK C.J., SALZAR R.S., LUCAS S.R., RAFAELS K.A., SHENDER B.S., PASKOFF G., *The temperature-dependent viscoelasticity of porcine lumbar spine ligaments*, Spine (Phila Pa 1976), 2007, 32, E436–42.

[13] NEUMANN P., EKSTROM L.A., KELLER T.S., PERRY L., HANSSON T.H., *Aging, vertebral density, and disc degeneration alter the tensile stress-strain characteristics of the human anterior longitudinal ligament*, J. Orthop. Res., 1994, 12, 103–12.

[14] STOJANOVIĆ A., OMEROVIĆ S., KRAŠNA S., PREBIL I., *Mechanical properties of human cervical spine ligaments: age related changes*, J. Biomech., 2012, 45(Supp 1), S611.

[15] CHAZAL J., TANGUY A., BOURGES M., GAUREL G., ESCANDE G., GUILLOT M., VANNEUVILLE G., *Biomechanical properties of spinal ligaments and a histological study of the supraspinal ligament in traction*, J. Biomech., 1985, 18, 167–76.

[16] MYKLEBUST J.B., PINTAR F., YOGANANDAN N., CUSICK J.F., MAIMAN D., MYERS T.J., SANCES A.JR., *Tensile strength of spinal ligaments*, Spine, 1988, 13, 526–31.

[17] PRZYBYLSKI G.J., CARLIN G.J., PATEL P.R., WOO S.L., *Human anterior and posterior cervical longitudinal ligaments possess similar tensile properties*, J. Orthop. Res., 1996, 14, 1005–8.

[18] BUTLER J., PINTAR F., YOGANANDAN N., MYKLEBUST J., REINARTZ J., SANCES A. JR., *Static and dynamic comparison of human cervical spinal ligaments*, Conf. Proc. IEEE Eng. Med. Bio. Soc. 1988, 1988, Vol. 2, 679–80.

[19] YOGANANDAN N., KUMARESAN S., PINTAR F.A., *Geometric and Mechanical Properties of Human Cervical Spine Ligaments*, J. Biomech. Eng., 2000, 122, 623–9.

[20] IVANCIC P.C., COE M.P., NDU A.B., TOMINAGA Y., CARLSON E.J., RUBIN W., PANJABI M.M., *Dynamic mechanical properties of intact human cervical spine ligament*, Spine J., 2007, 7, 659–65.

[21] LUCAS S.R., BASS C.R., SALZAR R.S., OYEN M.L., PLANCHAK C., ZIEMBA A., SHENDER B.S., PASKOFF G., *Viscoelastic properties of the cervical spinal ligaments under fast strain-rate deformations*, Acta Biomater., 2008, 4, 117–25.

[22] TROYER K.L., PUTTLITZ C.M., *Human cervical spine ligaments exhibit fully nonlinear viscoelastic behavior*, Acta Biomater., 2011, 7, 700–9.

[23] MATTUCCI S.F.E., MOULTON J.A., CHANDRASHEKAR N., CRONIN D.S., *Strain rate dependent properties of younger human cervical spine ligaments*, J. Mech. Behav. Biomed., 2012, 10, 216–26.

[24] SHARPE W.N., *Springer Handbook of Experimental Solid Mechanics*, Springer, 2008.

[25] MOON D.K., WOO S.L., TAKAKURA Y., GABRIEL M.T., ABRAMOWITCH S.D., *The effects of refreezing on the viscoelastic and tensile properties of ligaments*, J. Biomech., 2006, 39, 1153–7.

[26] CHENG S., CLARKE E.C., BILSTON L.E., *The effects of preconditioning strain on measured tissue properties*, J. Biomech., 2009, 42, 1360–2.

[27] QUINN K.P., WINKELSTEIN B.A., *Preconditioning is correlated with altered collagen fiber alignment in ligament*, J. Biomech. Eng., 2011, 133(6), 064506.

[28] NATALI A., PAVAN P., CARNIEL E., DARIO P., IZZO I., *Characterization of soft tissue mechanics with aging*, IEEE Eng. Med. Biol. Mag., 2008, 27, 15–22.

[29] PROVENZANO P.P., HEISEY D., HAYASHI K., LAKES R., VANDERBY R., *Subfailure damage in ligament: a structural and cellular evaluation*, J. Appl Physiol., 2002, 92, 362–371.

[30] PUTZ R., *The detailed functional anatomy of the ligaments of the vertebral column*, Ann. Anat., 1992, 174, 40–7.

[31] YOGANANDAN N., KUMARESAN S., PINTAR F.A., *Biomechanics of the cervical spine Part 2. Cervical spine soft tissue responses and biomechanical modeling*, Clin. Biomech. (Bristol, Avon), 2001, 16, 1–27.

[32] MOON D.K., ABRAMOWITCH S.D., WOO S.Y, *The development and validation of a charge-coupled device laser reflectance system to measure the complex cross-sectional shape and area of soft tissues*, J. Biomech., 2006, 32, 3071–3075.