Author’s response to reviews

Title: Function of ankle ligaments for subtalar and talocrural joint stability during an inversion movement – an in vitro study

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Author’s response to reviews:

Dear Editor, dear Reviewers,

We would first of all like to thank you for the time and effort you have invested in our manuscript and the helpful comments you have provided. We are also delighted that both reviewers commend us on our research project. Along with this we realize the specific comments of both reviewers with the aim to clarify open points and to further improve the quality of our manuscript. Having addressed all these issues in a detailed point by point response and in the revised manuscript, we are looking forward to your response.

Please find our detailed replies to your comments below. Text changes are highlighted in the mark-up version of the modified manuscript and referenced in our replies.

Reviewer 1: First of all I need to appreciate all the authors for the achievement in this study. Indeed, this is an interesting research, especially in the prevention and treatment of ankle injury. The Grammar is easy to understand and has written systematically. However, there are some that were be my concern, namely; why the number of samples is very small, so the statistical calculation becomes inadequate. I recommend that the biomechanical data of each cadaver be presented as a raw data, before it was calculated by mean value, so the reader can view the real value of biomechanical data of each cadaver. Another of concern was the age of human donors were very old, over 80 years old. There were needs to be an explanation of the condition of ligaments at this age, and their relevance to the results.

Reply: Thank you for your valuable comments that will be addresses point-by-point.
1) Number of subjects and statistical calculation:

We completely agree with you that the number of legs (n=8) included in the study is limited and may therefore affect interpretation of our results. Yet, referring to the very standardized experimental approach used and the observed moderate to large effect sizes we still believe that our conclusions hold true. Several aspects may be discussed when referring to the sample size available in the current study:

First, it is worth mentioning that the initial intention was to measure up to 14 legs as the specific research project presented here was part of a larger study focusing on different aspects of ankle joint stability. While it was possible to include all legs for the two other project parts, i.e., validation of another measurement device and biomechanical analysis of an operative technique, it immediately became evident, that not all legs will be measurable with the present ankle inversion simulation device. The reason for this was the high dynamical loading used in the present study approach, which was intentionally chosen to mimic higher angular rotation velocities than in past studies [1][2]. Screwing the bone-pins into fibula, calcaneus and talus and manually rotating the ankle joint dynamically, we observed a loosening of the screws and therefore decided to exclude them from further analyses in order to ensure validity and reliability of our data. The other project parts were not affected by this phenomenon as solely quasi-static/low-dynamic movements were applied. The possibility to use bone cements for improving fixation of the Kirschner wires as done in some comparable studies had been discussed during a pilot trial of our study. Unfortunately, this was finally not possible because it was clear that we should have the possibility to re-adjust or even re-screw bone-pins during the experiments. This was sometimes necessary when the bone-pins or the markers touched parts of the ankle deflection tester due to increasing laxity and therefore deflection of the ankle joint when ligaments were sectioned. Please be aware that this procedure did not influence our results as in these cases new static trials have been captured and used as a reference for the kinematic analysis.

In order to further enhance transparency we now included a brief statement in the methods section, describing the context of the current study approach more in detail. Specifically, we refer to the initial intention to include more subjects and the reason for retraction.

Second, please be aware that the initial intention for measuring 14 legs was not motivated by a previous minimum sample size estimation based on a power analysis. As we had the chance to get up to 14 legs in being part of a larger project, we finally aimed for as much legs to be included in our study as possible. Moreover, earlier studies with similar research questions and approaches have used comparable sample sizes[2][3][4][5]. For instance, in the study from Stacie et al. which focused on the effects of lateral ligament sectioning on the stability of the ankle and subtalar joint also eight cadavers were included[3].

Finally, given the small sample size in our study we used non-parametric tests, i.e., the Friedman test for the main effect and Bonferroni-Holm corrected Wilcoxon tests for post-hoc comparison between two conditions. Effect size calculation was performed using Kendall’s W, with values > 0.1 typically be interpreted as small effects, values > 0.3 as moderate and values > 0.5 as large effects. Referring to our data: In those cases with statistical significant main differences the
effect sizes ranged from 0.362 – 0.720 indicating always moderate or even large effects. Thus, we are still confident that our data provides valuable and reliable information regarding the stability of the ankle joint. Yet, as we agree that the sample size should definitely be considered when discussing the results, we have now added this point within the limitation section.

2) Presenting raw data of each subject

We welcome the request for presenting raw data of our measurement and we have modified the box plot figures displaying now also the raw data of each specimen. In addition, a table with individual data for all measure parameters will be included in the submission. Because this information is very detailed we have added it as a supplementary file, as we think it might be overwhelming for the reader directly in the manuscript.

3) Age of human donors (>80 years old) and the relevance of this to the results.

Thanks for this important issue. Indeed, for instance the study of Woo et al. confirmed that the ligament structure will get more and more lax by increasing age [6]. It is therefore very likely that the age of the specimen had an effect on the absolute mechanical properties and in consequence on related biomechanical parameters. However, the aim of the present study was not to report absolute ligamentous properties but rather to evaluate the relative contribution of the lateral ankle ligaments on individual joint stability. Assuming that age-related changes of ligament properties are occurring relatively homogeneously in each individual [6], especially if we consider ligaments that are closely related to each other and share a common function—age-specific effects would have solely minor influence on our results. Furthermore, it has to be considered that the literature dealing with the biomechanical function of ankle ligaments also contains high age cadavers as experimental subjects [7][3]. However, we completely agree that the age of the donors should be taken into consideration when interpreting the results. We therefore now address this point in the limitation section of our manuscript.

Reviewer #2: Thank you for the opportunity to review this manuscript. The authors describe a study where they seek to determine the specific influence of the three lateral ankle ligaments in stabilisation of the talo-crural and sub-talar joints, in neutral, plantar flexed and dorsiflexed orientations. This study extends from previous literature that have only reported the role of the lateral ankle ligaments in stabilisation of the entire ankle joint complex. The authors should be commended for their effort to undertake this technically difficult study. The manuscript is generally well written and clearly structured. I have a number of general and specific comments that are listed below.

General comments
Unfortunately I don't believe the authors can make conclusions about the mechanical role of specific ligaments acting about the sub-talar or talo-crural joints with the experimental approach employed here. The authors sequentially resected the ATFL, CFL and PTFL and measured changes in ankle joint kinematics for a given torque (stiffness). The order of resection was always constant. Unfortunately a major confounding limitation of this approach is the number of intact ligaments when testing each individual ligament. For the ATFL tests, there was still two lateral ankle ligaments intact, whereas for the PTFL tests, there were no lateral ankle ligaments intact. In order to determine the role of each ligament, the order of resection would need to be counter-balanced. This would enable a two way type analysis. The authors have addressed this in the limitations and I understand that this would take a much larger sample size. I also acknowledge the difficulty in obtaining suitable cadaveric specimens. However, this is a considerable limitation that confounds the primary findings of the study.

Reply: Thank you for this valuable comment, which is highly appreciated.

There were two dimensions of our former research purpose. One of them was to evaluate the biomechanics of the ankle joint complex and substructure of the ankle, i.e. the talocrural joint and the subtalar joint, when ligaments are sectioned, while the other was to detect the contribution of individual ligaments to joint stability.

For the first aim, we have confidence that our method allows us to draw a clear picture about physical phenomena of ligaments and their contribution to ankle joint stability in clinically relevant situations. The sectioning order followed the most common injury sequences of the target ligaments. The ATFL was sectioned first and then the CFL. This sequences was used because the ATFL is known to be the first or even the only ligament being injured[4], while the CFL is mostly injured along with the AFTL[8]. The PTFL was designed to be the last cut ligament, which is because only in rare cases the PTFL was damaged additionally[9]. Our sectioning sequence therefore reflects typical situations with one, two or three ligaments injured. Of course it has to be acknowledged that there are rather rare cases where for instance the CFL solely may be ruptured. Yet, most of the clinically relevant injury scenarios of lateral ankle ligaments are covered by our approach.

Referring to our second research aim, we have corrected the description of the individual ligament contribution in order to address our findings more clearly. We completely agree with you that it is hardly possible to make statements for instance about the function of the PTFL solely while the ATFL and the CFL are cut. Therefore, we have modified our manuscript, i.e. preliminary the aim and the discussion sections. It is now more clearly expressed that the changes observed when cutting the CFL or the PTFL have to be interpreted under the condition that already one or two ligaments are sectioned. Thus, keeping the general structure of our discussion we now express more clearly that changes in ankle joint stability cutting for example the CFL always refer to the situation when the ATFL was cut before when we discussed the talocrural joint and the global ankle joint complex.

Anyways, some of our observations can be related to an isolated effect of specific ligaments. For example, cutting the ATFL solely did not lead to any substantial changes in the dorsiflexed
position. This can be explained by the anatomical orientation of the ATFL which should be loose during ankle dorsiflexion. In contrast, cutting the CFL in addition increases the ankle inversion. This indicates that the CFL should play a major role in stabilizing the ankle joint relatively independent from the integrity/rupture of the ATFL.

In conclusion, we believe that the modified version of the manuscript helps readers to have a better understanding about the ligament functional role of joint stability from biomechanical view.

2. Statistics - It is unclear how the statistical approach was undertaken. Was this a two way analysis (Eg Ankle joint position x number of intact ligaments)? Was the non-parametric approach implemented due to lack of normality in data? How were effect sizes calculated?

Reply: Thank you for this comment. Considering the small sample size in our study, we did directly use a non-parametric test approach. Specifically, a Friedman test was used to test for the main statistical effects of sectioning conditions (Intact, ATFL cut, ATFL+CFL cut, ATFL+CFL+PTFL cut) in each ankle positions separately (neutral position, 10° plantarflexion, 10° dorsiflexion). If the result of the Freidman test indicated significant differences between ligament conditions, a Bonferroni-Holm corrected Wilcoxon test was applied as a post-hoc test to determine the difference between two separate conditions. Moreover, we used Kendall’s W to calculate the effect size of the main effects between ligament conditions with values > 0.1 typically be interpreted as small effects, values > 0.3 as moderate and values > 0.5 as large effects. Accordingly, we modified the statistics section of the manuscript and added the definition of the effect size calculation to clarify our statistics approach.

4. Discussion - It might be appropriate to have some discussion as to the ecological validity of these findings. Are the torques and loading rates similar to this that may be experienced during walking, running or cutting?

Reply: Thank you for your valuable suggestion. Surrogating for loading rate, the angular velocity might be considered. In our study, the mean angular velocity of our inversion motion task is 64.1°/s ± 11.8°/s. This is comparable to previously reported basic movements such as running (maximum ankle inversion velocity: 85.1°/s), cutting (maximum ankle inversion velocity: 37.2°/s) and jumping (maximum ankle inversion velocity: 22.5°/s)[10]. Yet, it has to be mentioned that situations with ankle sprains are associated with significant higher rotational velocities. Inversion velocities from 200°/s to 300°/s are typically observed when for instance simulating ankle sprain situations in the standing subject in-vivo with tilt platforms[11]. Therefore, the angle velocity in our motion task reflect typical non-injury locomotion task quite well but only reach 25% of ankle ligamentous sprain simulation. Thus, a specific discussion of our motion task design was added to our manuscript in order to keep our study rigorous and delivery precise findings to readers.
Specific comments

Abstract
Line 28 - I don't think "kinematical" is a word. Perhaps change to "a motion capture system recorded kinematic data from"

Reply: Thank you for your comment. We changed it accordingly.

Introduction
Line 48 - please remove the 's' from 'inversions'

Reply: We appreciate your comment. We changed it accordingly in the text.

Line 61 - 'precisely' seems out of place here.

Reply: Thank you for your remark. We provided changes in the text accordingly.

Line 87 - Please change 'Second' to 'secondly'

Reply: Thank you for your comment. We changed it accordingly in our manuscript.

Methods
Line 122 - change 'applying' to 'application of'

Reply: Thank you for your comment. We modified it accordingly.

Line 140 - Was only 1 motion capture camera used? I presume this is an error?

Reply: Thank you for your observation. We added the correct number of cameras.

Line 185 - Please clearly outline the statistics - Was this a two way approach? How was effect size calculated?

Reply: Thank you for your comment. We modified the statistics session to clarify our statistics approach.
Discussion

Line 268 - The magnitudes of angular rotations are very small. Are the authors confident that the measures are within the resolution of the motion capture system?

Reply: Of course, the resolution in space and time of the measurement approach should be well selected regarding the motion being analysed. Yes, we are confident that our motion capture approach is highly capable in providing sufficient accurate data under the current experimental conditions. The system used (Vicon Motion System, UK) consisted of 6x MX-40 cameras and 5 MX-3+ cameras with an optical sensor resolution of 4 mega pixels and 0.3 mega pixels, respectively. Under the current measurement conditions, i.e., cameras were placed in average 2.5 meter from the specimen, the resolution of one pixel is around 1.4 mm x 1.4 mm. Considering that the centre of a 6 mm marker, being composed of multiple pixels, was used for further calculations the accuracy would approximate 0.37 mm in MX-40 camera and 0.51mm in MX-3+ camera. The markers representing the coordinate systems were placed on Kirschner wires with a length of about 5 cm. Using basic trigonometric calculations a change in the markers’ relative coordinates of 1.2 mm (given a maximal error of 0.51mm for the low resolution cameras) from two perpendicular Kirschner wires, the angle will change less than 0.0001° in 2 dimension. As this potential error is much smaller that our observed differences between different test conditions, we strongly believe our measurement approach was highly sufficient in detecting changes appropriately.

Line 287 - 'Locked' is a poorly defined term. Perhaps 'stiffened' would be more mechanically appropriate?

Reply: We appreciate your comment. We changed it accordingly in our manuscript.

Line 290-294 - This suggestion highlights the potential importance of a well defined bone coordinate system. Did the authors calculate a mean or instantaneous helical axis for these bones or joints? This may help to shed some light on changes in kinematics with ligament resection

Reply: : Regarding the data analysis procedure we first have to clarify that we didn’t use a helical axis approach for the calculation of joint angles. We rather used a Joint Coordinate System (JCS) approach as suggested by the International Society of Biomechanics[12]. This approach first establishes a cartesian coordinate systems for each of the two segments based on anatomical landmarks. Then, the JCS is established based on the two cartesian coordinate systems with two axes being fixed to one of the segments and the third axis is a floating axis. Regarding the ankle joint, the definition of the JCS is proposed as follows:

Figure 1 Proposal of the JCS for the ankle joint with e1 and e3 being the body-fixed axes and e2 (=inversion/eversion) being the floating axis (Wu et al. 2002).
We followed this recommendation as a comparison of different calculation methods (JCS, Euler angles, helical axis) for detecting ankle and subtalar joint instability revealed that the JCS might be an appropriate approach[13]:

“The Euler angles and JCS results show consistency during supination as significant differences were found after the CFL was cut around the inversion/eversion axis and after the ATFL was damaged around the interior/exterior rotation axis but the helical axis angle of rotation decomposition showed the opposite. Therefore, using the helical axis parameters to analyze kinematics at the ankle joint should be restrained to plantar/dorsiflexion motion.” (see Choisne et al. 2011, p. 51).

Regarding the question if mean or instantaneous axes (in our approach this would refer to the floating inversion-eversion axis) were calculated: In the JCS approach instantaneous, i.e., frame-by-frame = 5ms, changes in floating axis are used to reflect the anatomical situation most appropriately.

Aiming to describe our calculation approach more clearly, we have now enlarged our description in the methods section.


