Torsion Constants and Virtual Mechanical Tests are Valid Image-Based Surrogate Measures of Ovine Fracture Healing

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Abstract

In large animal studies, the mechanical reintegration of the bone fragments is measured using postmortem physical testing, but these assessments can only be performed once, after sacrifice. Image-based virtual mechanical testing is an attractive alternative because it could be used to monitor healing longitudinally. However, the procedures and software required to perform finite element analysis (FEA) on subject-specific models for virtual mechanical testing can be time consuming and costly. Accordingly, the goal of this study was to determine whether a simpler image-based geometric measure—the torsion constant, sometimes known as polar moment of inertia—can be reliably used as a surrogate measure of bone healing in large animals. To achieve this, postmortem biomechanical testing and microCT scans were analyzed for a total of 33 operated and 20 intact ovine tibiae. An image-processing procedure to compute the attenuation-weighted torsion constant from the microCT scans was developed in MATLAB and this code has been made freely available. Linear regression analysis was performed between the postmortem biomechanical data, the results of virtual mechanical testing using FEA, and the torsion constants measured from the scans. The results showed that virtual mechanical testing is the most reliable surrogate measure of postmortem torsional rigidity, having strong correlations and high absolute agreement. However, when FEA is not practical, the torsion constant is a viable alternative surrogate measure that is moderately correlated with postmortem torsional rigidity and can be readily calculated.

Introduction

Fracture healing is a complex physiological process, and the assessment of fracture healing progress is important both for examining clinical fractures and evaluating outcomes in preclinical studies. In preclinical studies of long bone healing, postmortem biomechanical testing is the gold-standard method for assessing the mechanical progression of fracture repair. In large animals, the most common postmortem test is a torsion test,[1–4] but in murine studies, bending tests are also used.[5–8] One drawback of these biomechanical testing methods is that they can only be completed after sacrifice, so they are not useful for longitudinal monitoring, and they do not translate to a clinical setting. Imaging-based methods are a promising alternative, but the determination of bony union from conventional radiographs is not well defined and often subjective.[9] Therefore, there is a need for translational tools that can assess the structural progress of bone repair in living animals and humans.

Finite element analysis (FEA) is increasing in prevalence as tool for understanding fracture healing. Subject-specific models built from computed tomography (CT) scans can capture both the complex geometries and mineralization gradients in and around the fracture healing zone. To develop the models, bones are segmented from the CT scans and converted into meshes using voxel-based[10] or smooth geometry-based[11] methods. Density-dependent scaling laws are used to convert the radiodensity (gray value) in each voxel of the scan to a Young's modulus, E. Finally, boundary conditions are applied to the models to mimic the desired biomechanical test. A summary biomarker such as torsional rigidity is then estimated from the solved model. Recent studies have shown that image-based virtual mechanical testing from CT scans can closely replicate physical biomechanical testing.[4,12] CT-based structural rigidity assessment has also shown promise for fracture risk assessment in metastatic lesions.[13] However, a notable disadvantage of this method is that the process of model-building can be time consuming and may require the researcher to have sophisticated skills and specialized software access.

As a result, in some applications it would be desirable to have a simple surrogate biomarker for the load-bearing capacity of a healing bone that can be derived directly from imaging data without the need for full finite element analysis.[14,15] One promising mechanical biomarker is the torsion constant, sometimes also called the *polar moment of inertia*. In a torsion mechanical test, a linear regression is used to fit the slope of the torque (T) versus angle of twist (Φ) curve. This slope is the torsional stiffness. Torsional stiffness can also be defined as the product of the geometrical torsion constant (J) and shear modulus (G), divided by the length of the test segment (L). Geometrical torsion constants have been previously computed using imaging data for intact human cadaver bone,[16] evaluated theoretically in the context of fracture healing,[17,18] and reported as summary biomarkers of long-bone healing in rodents and goats.[19] In mice, the polar moment of inertia is positively correlated with the callus volume and tends to decrease over time as the bone remodels.[20] However, a recent systematic review of methods for assessing bone union in animal studies questioned the strength of association between polar moment of inertia and the outcome measures from physical mechanical testing.[21] Furthermore, the use of a polar moment or torsion constant parameter to characterize healing in large animals has not been explicitly validated.

Accordingly, the technical objective of this investigation was to develop an open-source numerical method for computing the torsion constant of a healing long bone from microCT images of sheep. The goal of the research was to assess the reliability of this measure as a surrogate for postmortem biomechanical data and to compare the results with virtual mechanical testing using FEA. Meanwhile, find the best predictors of postmortem biomechanical testing in a large animal model. The hypothesis of the study was that the torsion constant measured from effective polar moment of inertia, J_{eff}, is a reliable predictor of postmortem torsional rigidity, GJ, in osteotomized ovine tibiae.

Methods

A schematic overview of the overall study design is presented in Fig. 1.

Animal Specimen Information

Forty-four adult female Swiss alpine sheep (2-3 years old, weighing 59–87 kg) were used in three prior research projects using three distinct tibial osteotomy models supported with medial plating (Fig. 1A). In total, there were 33 operated limbs and 20 intact control limbs that had both mechanical test data and microCT scans available for analysis in this study. The complete details of these experiments have been previously reported[4] and are summarized here in brief. Group 1 consisted of seven animals with a 3mm gap defect stabilized with a 12-hole stainless steel plate (broad straight veterinary 3.5 mm locking compression plate (LCP), 159mm in length, with 3.5 mm bicortical screws; DePuy Synthes). Group 2 consisted of 18 animals with a 3mm gap defect stabilized with a six-hole titanium plate (broad 4.5/5.0 mm LCP, 115.8mm in length, with 5mm bicortical screws; DePuy Synthes). Sheep in Groups 1 and 2 were sacrificed 9 weeks after surgery. Group 3 consisted of eight animals with a 17mm defect augmented with autografts and stabilized with a 13-hole stainless steel plate (broad straight veterinary 3.5 mm LCP, 172mm in length, with 3.5 mm bicortical screws; DePuy Synthes). Sheep in Dataset 3 had slower healing and were sacrificed 12 weeks after surgery. All experiments were conducted at the Musculoskeletal Research Unit in Zürich, Switzerland, according to the Swiss laws of animal protection and welfare and authorized by the local governmental veterinary authorities (License No. ZH 183/17).

Micro-Computed Tomography (microCT) Scanning

After animal sacrifice, the operated tibiae were excised, stripped of soft tissue, and all hardware was removed, taking care not to disrupt the callus or periosteum. Samples were wrapped in saline-soaked gauze and microCT scanned using an XtremeCT II Micro-CT scanner (Scanco Medical AG, Bruettisellen, Switzerland) with an X-ray voltage of 68 kVp and X-ray current of

1470 µA. The resulting scans had an isotropic resolution of 60.7 µm. Hounsfield Units [HU] were converted to radiodensity [mgHA/cm^3] using data from a hydroxyapatite phantom calibration scan.

Mechanical Testing

Physical torsion tests were performed on all included samples using a custom-made fixture on an Instron E10000 electrodynamic testing machine (Instron). Mechanical tests were performed by quasi-statically preloading the limb with 5N axial load, which was held for the entire test, and then applying internal rotation at 5° per minute. Torque and rotation angle were continuously recorded during the test. Biomechanical torsional rigidity (experimental GJ) was calculated as a linear regression of the torque-angle curve multiplied by the specimen gauge length, which was measured after embedding and ranged from 140 to 160 mm. Additional details on the experimental procedure were previously reported.[4]

FEA Modeling & Virtual Torsional Rigidity Testing

Finite element models were built from the microCT scans of all intact and operated limbs following our previously published procedure.[12] Images were first down-sampled to 400 µm isotropic resolution and then segmented using Mimics Innovation Suite (vs 21.0; Materialise, Plymouth, MI) to identify bone and callus. Callus volume, V, was recorded from the segmented mask. A quadratic tetrahedral mesh was applied to each sheep model with a maximum surface edge length of 1 mm and a maximum interior edge length of 1 mm. These image-based models require assignment of elementwise material properties based on local density data within the scan. In prior research, we established two distinct scaling equations for assigning the density-dependent Young's modulus of ovine tibial cortical bone with and without callus (Fig. 1C). In the first method, we optimized a scaling equation for cortical bone using data from intact ovine tibiae, resulting in a linear function[4]:

$$E = 10225 \times \rho_{QCT} \quad (1)$$

where E is Young's modulus [MPa] and ρ_{QCT} is the phantom-calibrated radiodensity [mgHA/cm^3]. To account for the distinct mechanical contributions of hard and soft callus in early fracture repair, we also developed a piecewise-defined dual-zone material model defined as follows:

$$E = \begin{cases} E_{sc} & \rho_{QCT} < \rho_{cut} \\ 10225 \times \rho_{QCT} & \rho_{QCT} \geq \rho_{cut} \end{cases} \quad (2)$$

where E_{sc} is Young's modulus [MPa] of the soft callus and ρ_{cut} is the density cutoff [mgHA/cm^3] that differentiates between soft callus and hard callus. In a previous validation study, we selected E_{sc} = 50 MPa and ρ_{cut} = 665 mgHA/cm^3 to produce good agreement between physical and virtual mechanical tests of the operated tibiae.[12]

Virtual mechanical testing was included in this study as a candidate predictor of postmortem biomechanical torsional rigidity, experimental GJ, and for comparison to the torsion constant. The simulated torsion test produces a summary parameter, the virtual torsional rigidity (VTR):

$$VTR = \frac{ML}{\Phi} \quad (3)$$

where M is the calculated moment reaction, L is the working length of the test segment, and Φ is the applied angle of twist. In our prior validation studies, virtual mechanical testing with a linear material assignment law (Equation 1; VTR_{linear}) achieved close correspondence between physical and virtual torsion tests of intact ovine tibiae.[4] For osteotomized tibiae, the dual-zone material assignment law (Equation 2; VTR_{dual}) that represents both the hard and soft mechanical characteristics of callus outperformed single-zone material modeling for the operated limbs.[12] Both intact and osteotomized limbs are included in this study for assessment of the torsion constant, so both the VTR_{linear} and VTR_{dual} data are included in this analysis.

Evaluation of Torsion Constant from microCT

The effective polar moment of inertia J_{eff} was calculated as an approximation of the effective torsion constant for each limb using an image analysis technique in MATLAB (2021a; The MathWorks, Inc., Natick, Massachusetts, USA). An open-source code for processing microCT scans to calculate J_{eff} using the method described below has been posted on GitHub and is free to use.[22]

The first step for calculating the torsion constant is to define the region of interest (ROI) in each scan. A standardized segment length was chosen by measuring all of the callus lengths from the finite element models. The maximum segment length was recorded and utilized as the length of the ROI for all other sheep (Fig. 1B). This ensured that the entire callus of every scanned sheep was included in the ROI for image analysis.

Within each scan, we performed an automated segmentation on each 2D tomogram positioned between the proximal and distal bounds of the selected ROI. The method was modified from a previous our previous study using density-based segmentation.[23] First, the soft tissue region was excluded using connected-component labeling. Noise was then reduced by applying a median filter. Next, the original dataset was re-thresholded and masked with the detected boundaries of the bone-callus region. Dilation and erosion operations were then used to determine the bone and callus boundary which were then refined by minimization of spline energy to achieve smoothness of this detected tissue boundaries for each sheep. Finally, this segmentation procedure identified the cortical/callus outer boundary, or if an intact bone, the area enclosed by the cortical bone periosteal surface (Fig. 1D). The attenuation-weighted in-plane polar moment of inertia, J_i, was then calculated for each slice:

$$J_i = \sum_{k=1}^{N} r_k^2 A \frac{\rho_k}{\rho_{max}} \quad (4)$$

where A is the in-plane area of each of the N included voxels, r_k is the distance from the k^{th} voxel to the centroid of all included voxels in the i^{th} transverse slice in a scan, ρ_k is the radiodensity of pixel k, and ρ_{max} is the maximum radiodensity within scan. The effective torsion constant of the entire ROI was then computed numerically using:

$$J_{eff} = L\left(\int_0^L 1/J(z)\, dz\right)^{-1} \quad (5)$$

where L is the axial segment length of the ROI and $J(z)$ is the polar moment of inertia J_i of the cross-section located at the longitudinal position z.

In addition to attenuation-weighting, a variable global threshold was used to distinguish mineralized tissue from unmineralized and poorly mineralized tissue, which in turn dictated which voxels would be considered for the calculation of J_i. In a previous study, we showed that early-stage callus is comprised of both hard and soft regions and that hard zones are what confer the organ-level torsional rigidity.[12] Here, we adopted a similar approach and defined a cutoff density, ρ_{cut}, that would exclude the voxels corresponding to the medullary canal, callus voids, and some soft callus from the J_i calculation (Fig. 1D). For the baseline case, we used a cutoff of 665 mgHA/cm^3 based optimized cutoff for distinguishing between hard and soft callus from our previous validation study of virtual mechanical testing.[24] We also performed an optimization on ρ_{cut} to identify the best threshold for distinguishing mineralized tissue from unmineralized and poorly mineralized tissue when calculating the torsion constant (Fig. 1E) by maximizing the correlation coefficient obtained between J_{eff} and torsional rigidity from physical testing (GJ).

Statistical Analysis

Statistical analyses were generated using MATLAB 2021a Statistics and Machine Learning Toolbox (The MathWorks, Inc., Natick, Massachusetts, USA). Linear regressions were used to determine the strength of the linear relationship between several outcome variables (J_{eff}, VTR_{linear}, VTR_{dual}, and callus volume V) and the experimental GJ (Fig. 1F). Correlation coefficients were interpreted as follows: poor $r \leq 0.2$, fair $r \leq 0.6$, moderate $0.6 < r < 0.8$, or strong $r \geqslant 0.8$.[25]

Results

Performance of Image-Based Predictors of Postmortem Biomechanics

Scatter plots in Fig. 2 show the coefficient of determination between all pairs of variables (V, VTR_{dual}, VTR_{linear}, J_{eff} and GJ) measured across the specimens. All correlations were statistically significant ($p < 0.0001$) except the correlation between callus volume, V, and VTR_{dual} ($p = 0.0024$) and V and GJ ($p = 0.0032$). There was one strong correlation between VTR_{linear} and J_{eff} ($R^2 = 0.96$, $p < 0.0001$). In general, FEA-based measurements of torsional rigidity (VTR_{linear} and VTR_{dual}) showed strong and significant correlations with GJ ($R^2 \geq 0.63$, $p < 0.0001$). Here, VTR_{dual} was calculated at the previously optimized density cutoff ρ_{cut} = 665 mgHA/cm^3 to differentiate between hard and soft callus. The image-based torsion constant J_{eff} showed a moderate correlation with experimental GJ ($R^2 = 0.55$, $p < 0.0001$), while callus volume, V, showed a weak correlation with experimental GJ ($R^2 \leq 0.26$, $p = 0.0024$).

The relationships between measures that produced moderate-to-strong correlations with both experimental GJ (J_{eff}, VTR_{linear}, and VTR_{dual}) are illustrated in Fig. 3 for all operated tibiae. Application of the baseline global threshold value ρ_{cut} = 665 mgHA/cm^3 resulted in removal of the lowest-density voxels of the callus from the attenuation-weighted J_i calculation (Fig. 3A). For virtual mechanical testing with the linear material assignment law (Equation 1), elements corresponding to these regions were treated as low-density bone. In the dual-zone material assignment law (Equation 2), these low-density elements were assigned soft callus properties (E_{sc} = 50 MPa). The torsion constant, J_{eff}, exhibited a strong correlation with VTR_{linear}, but only a moderate correlation with GJ and VTR_{dual} (Fig. 3B/D). Notably, VTR_{dual} had a slightly superior correlation with GJ compared to VTR_{linear} at ρ_{cut} = 665 mgHA/cm^3 (R^2 = 0.67 vs. 0.64; Fig. 2), with substantially improved absolute agreement ($RSME$= 0.157 vs. 0.451).

Factors Influencing Torsion Constant Performance

The effect of including a variable global threshold ρ_{cut} in the torsion constant calculation was evaluated by sweeping the cutoff between 0 and 1200 mgHA/cm^3 and recalculating the correlations between J_{eff} and the torsional rigidity measurements (GJ and VTR) in three groups of animals: intact only (N = 20) (Fig. 4A); operated only (N = 33) (Fig. 4B); and mixed intact and operated (N = 53) (Fig. 4C). Note that in this analysis, VTR_{linear} does not depend on ρ_{cut}; only VTR_{dual} and J_{eff} depend on the soft callus cutoff density.

In the intact limbs (Fig. 4A), changing the global threshold had only a marginal effect on the correlation between J_{eff} and experimental GJ. J_{eff} also had a very strong correlation with both VTR measures across almost the full density cutoff range. There were very few low-density voxels present within the cortical walls and the attenuation-weighting procedure in Equation 4 for the calculation of J_i in each 2D tomogram was sufficient to eliminate the contribution of the void voxels within the medullary canal.

In contrast, for the operated group (Fig. 4B), the correlations between J_{eff} and torsional rigidity (GJ and VTR) were highly sensitive to the global threshold, ρ_{cut}. Up to a density cutoff of 800 mgHA/cm^3, J_{eff} was a moderately strong predictor of GJ and was not sensitive to ρ_{cut}, but above this level, the correlation between J_{eff} and GJ dropped precipitously. Similarly, J_{eff} was strongly correlated with VTR_{linear} at low values of ρ_{cut} and weakly correlated at high values. Interestingly, the correlation between J_{eff} and VTR_{dual} exhibited non-monotonic behavior, with both measures being sensitive to ρ_{cut}, but at different thresholds. As ρ_{cut} increased, more voxels were excluded from the torsion constant calculation and their corresponding elements were assigned to the soft tissue group in the finite element models. Although these effects are conceptually similar, the VTR_{dual} measure decreased more quickly than J_{eff} at high values of ρ_{cut}.

Combining all limbs, both operated and intact, drastically reduced the ability of the torsion constant to predict experimental GJ (Fig. 4C). As in the operated-only group, the mixed group

showed non-monotonic behavior of Pearson's correlation coefficient between J_{eff} and experimental GJ, as well as between J_{eff} and VTR_{dual} as a function of ρ_{cut}. When ρ_{cut} increased from 500 mgHA/cm^3 to 800 mgHA/cm^3, the absolute value of VTR_{dual} dropped drastically (52% average reduction) while the value J_{eff} had only marginal change (4% average reduction). When the ρ_{cut} further increased to 1000mgHA/cm^3, the value of J_{eff} showed a substantial drop and the correlation coefficient increased again to 0.81. Notably, this strong correlation between J_{eff} and VTR_{dual} at the highest values of ρ_{cut} was achieved with inaccurately low predictions of rigidity and torsion constant in the operated limbs.

Finally, for the operated animals only, we evaluated the performance of J_{eff} as a surrogate marker for GJ compared to the results of virtual mechanical testing using the dual-zone material model (Fig. 5). For the lower range of density cutoffs up to 600 mgHA/cm^3, VTR_{dual} was more strongly correlated with experimental GJ than J_{eff}. At higher cutoffs, the ability of both J_{eff} and VTR_{dual} to predict variations in experimental GJ was diminished. When density cutoffs were fine-tuned to achieve optimal correlations, VTR_{dual} had a strong association with physical torsional rigidity (R^2_{max} = 0.75), but the effective torsion constant could only achieve a moderate correlation with physical torsional rigidity (R^2_{max} = 0.61). The virtual torsional rigidity with dual-zone material assignment also provided good absolute agreement with the measured torsional stiffness from postmortem torsion testing (Fig. 5B).

Discussion

The goal in many preclinical fracture healing studies is to measure the healing progress of the fractured bone using a variety of tools. A key takeaway of this study is that when properly employed, both the torsion constant and the virtual torsional rigidity are useful imaging-based biomarkers of long bone healing biomechanics in large animals. This work resolves uncertainty in the literature regarding the strength of association between polar moment of inertia and the outcome

measures from physical mechanical testing and the lack of validation of polar moment or torsion constant as a surrogate measure of fracture healing in large animals. Our results showed that virtual mechanical testing, especially with appropriate material modeling of soft callus, is the most reliable non-destructive replacement for physical mechanical testing, but it is not the only useful tool. Each method we tested has strengths and weaknesses that a researcher needs to understand for interpreting results, or that may guide the selection of one measure over the other.

A clear advantage of subject-specific finite element analysis is that it can reliably replicate postmortem physical biomechanical testing of intact and operated ovine tibiae. Virtual mechanical testing produced results that were strongly correlated with experimental GJ and had good absolute agreement. Compared to the torsion constant, VTR_{dual} had the additional advantage of being a direct surrogate measure for GJ, with the same units [Nm^2/°], and the same intuitive physical meaning (slope of the torque-angle curve, adjusted for specimen length). Unsurprisingly, VTR_{linear} and J_{eff} had the strongest correlation with each other because both account for geometry a linear scaling contribution from radiodensity. Compared to VTR_{linear}, VTR_{dual} has the advantage of better absolute agreement with experimental GJ, making it a more reliable surrogate measure when FEA can be performed.[24] However, the steps involved in construction, analysis, and validation of FE models of healing bones are complex.[24] A successful subject-specific modeling procedure starts with segmentation of the areas of interest and mesh generation of the segmented areas. Density-dependent material properties must be correctly applied and loads and boundary conditions carefully matched to the experimental conditions to enable model validation using *in vitro* data. These procedures require the use of specialized software that can be expensive to license and know-how that can take considerable time to develop. For these reasons, even though virtual mechanical testing produced the most reliable surrogate measure for experimental GJ, adding this work may not be an expedient choice for all research designs.

297 In contrast, J_{eff} can be calculated directly from the microCT scans using the MATLAB
298 code we have shared. This method does not require access to commercial CT scan processing or
299 structural analysis software and can be readily translated to other languages by a researcher with
300 general coding skills. Comparatively, J_{eff} was not as reliable as VTR_{dual} for predicting GJ, but it
301 still had a moderately strong correlation with experimental GJ when considering intact and operated
302 limbs separately. A notable limitation of J_{eff} is that it is an indirect surrogate for experimental GJ,
303 having different units [mm^4] and a non-intuitive physical interpretation for researchers without a
304 mechanics background (geometry-associated resistance to torsional distortion). The torsion
305 constant also performed notably poorly at predicting experimental GJ when intact and operated
306 tibiae were combined (Fig. 4C).

307 This study showed that while VTR and J_{eff} both have utility as candidate measures of bone
308 repair, they must be interpreted with caution when designing future studies, particularly when
309 imaging-based methods are to be used for longitudinal monitoring of fracture healing. To illustrate
310 some of the potential pitfalls with each measure, Fig. 6 shows the hypothetical progression of
311 secondary fracture healing in a representative transverse fracture with idealized schematic time-
312 history curves for J_{eff} and VTR in two fractures: one quick healer and one slow healer. Initially,
313 before any healing has occurred, J_{eff} values are close to the intact state because the osteotomy
314 represents a relatively small defect along the length of the tibia. In contrast, VTR is initially zero
315 because the bone is totally non-united. As the callus grows, J_{eff} increases to a maximum value,
316 then it decreases due to remodeling, eventually returning a value equivalent to an intact bone. In
317 contrast, VTR increases gradually, eventually achieving a steady-state value equivalent to the intact
318 bone.

319 Comparing these measures for a single timepoint in early healing, both J_{eff} and VTR
320 detect that the faster healer has superior healing (compare points N and P in Fig. 6B/C). As a

longitudinal outcome measure, J_{eff} could detect the growth of callus (increase from point M to N) or remodeling of callus (decrease from point N to O). However, one notable challenge is that early-stage healing, delayed healing, and late-stage remodeling could all have similar J_{eff} values, which may be close to that of intact bone (points M, P, and O), even though their biological and structural status would be profoundly different. In contrast, VTR would always detect lower values in early or delayed healing with limited callus (points M and P). The challenge with VTR is that after the fracture has bridged, rigidity would remain nearly constant over time (points N and O) even while substantial remodeling changes are ongoing.

Considering these challenges, we suggest that J_{eff} may be most useful for comparing between groups at matched early timepoints when the callus is increasing in volume and density. A study design factor that may indicate against the use of J_{eff} would be the inclusion of late timepoints in a quick-healer group for comparison to an atrophic nonunion group because their torsion constants may be too similar (close to intact bone). Alternatively, the full finite element analysis would be required to compare the structure healing between substantially different cohorts or to test for longitudinal changes at widely varying healing times. However, VTR would not be useful in a study focused on late-stage remodeling when no additional changes in rigidity are expected.

A discussion of the strengths and limitations of J_{eff} and VTR must also come with a caution about the common and potentially incorrect assumption that callus volume is a reliable surrogate measure for fracture healing biomechanics. In this study, the torsion constant and torsional rigidity measures all tended to increase with larger callus volumes, but the association was not strong. In fact, callus volume had only a weak correlation with the biomechanical test results (GJ) and VTR_{dual} in these sheep. One reason for this observation is our inclusion of both 3-mm and 17-mm osteotomies with autograft. The large-defect animals were delayed in their healing but had a larger healing zone. However, even within the 3-mm defect groups there were variations

in callus volume between animals that did not necessarily indicate superior or inferior rigidity. For ovine osteotomies at this stage of healing, inhomogeneity in callus mineralization produces variations in measured rigidity that are not captured by measuring callus volume alone.

This work is not without limitations. First, the torsion constant was analyzed in the original image resolution (60.7 microns) while the FEA models were developed from scans that had been downsized to 400-micron resolution to avoid intractably large meshes. Additionally, we used a numerical integration of the in-plane moment of inertia to estimate the torsion constant. In a cylindrical prism, the torsion constant equals the polar moment of inertia. This assumption is not true for noncircular geometries, which experience warping under torsion. In some simple noncircular geometries, a Laplace equation with Neumann boundary condition can be solved analytically to derive the warping function.[26,27] Unfortunately, an explicit analytical solution for the warping function does not exist except for very simple geometries. For more complex geometries such as long bones, a numerical approach such as the finite element method, must be used to correctly calculate the torsion constant accounting for warping effects. The image-based effective torsion constant (J_{eff}) reported here neglects warping, but this assumption is warranted under the small-strain conditions represented by the mechanical tests.

Conclusion

In summary, this study showed that image-based assessments can be used as a predictors of whole-bone biomechanical properties in ovine tibial osteotomies. Virtual mechanical testing is the most reliable surrogate measure of postmortem torsional rigidity. When FEA is not practical, the attenuation-weighted torsion constant is a viable alternative that is moderately correlated with postmortem torsional rigidity and can be calculated without requiring specialized software or know-how. For best results, both torsion constant and torsional rigidity measures should be implemented with a global threshold to distinguish between soft and hard callus based on radiodensity.

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Figure Captions

Figure 1 – This flowchart outlines the entire study. A) *In vivo* data was derived from three ovine osteotomy studies. B) MicroCT scans were segmented to identify the region of interest (ROI) at the midshaft. A variable density cutoff (ρ_{cut}) was defined to distinguish between mineralized tissue and soft callus or background pixels. C) The density cutoff was varied and the effective torsion constant (J_{eff}) calculated for all intact (N = 20) and osteotomized (N = 33) tibiae. D) Virtual mechanical testing was also performed using the finite element method with two options for assigning elementwise material properties: a linear material model and a piecewise dual-zone material model. E) Postmortem biomechanical testing was used to measure the physical torsional rigidity (GJ) of all samples. F) Correlation analysis was used to assess which imaging-based biomarkers are good predictors of experimental GJ.

Figure 2 – Correlation plot of all variables for operated tibiae. Each data point represents one osteotomized ovine tibia (N = 33 total). Histograms of all the variables (Callus volume, VTR_{dual}, VTR_{linear}, J_{eff} and experimental GJ) are shown along the main diagonal. Plots in the off diagonal show the strength of association (R^2) between all pairs of variables in this study. Green correlations are very strong, purple correlations are moderate or strong, and orange correlations are weak.

Figure 3 – A) Section view of the callus for one animal showing image thresholding at the baseline density cutoff ρ_{cut} = 665 mgHA/cm^3 to differentiate between hard and soft callus. B) The finite element analysis (FEA) with linear material assignment treats all elements as bone of varying density, C) while the dual-zone model assigns soft-tissue properties to low-density elements. B/C/D) Scatter plots show the strength of association between the computed torsion constant, J_{eff}, and torsional rigidity measured in postmortem biomechanical testing and by virtual torsion testing with the linear and dual-zone material models for all operated tibiae.

Figure 4 – A) In the intact limbs, J_{eff} was strongly correlated with torsional rigidity and was not sensitive to cutoff density. B) For the operated limbs, J_{eff} was moderately-to-strongly correlated with experimental GJ and VTR at low density cutoffs. As density cutoff increased, mineralized voxels were increasingly excluded from the J_{eff} calculation, leading to worsening correlations. C) Combining the intact and operated groups led to poor correlations between J_{eff} and experimental GJ. Example correlations in (C) show that at high values of ρ_{cut}, the J_{eff} vs. VTR_{dual} correlation coefficients increased, although the predictions of rigidity and torsion constant in the operated limbs became artificially low.

Figure 5 – A) Considering operated animals only, for the lower range of density cutoffs, VTR_{dual} was more strongly correlated with GJ than J_{eff}. At higher cutoffs, the ability of both J_{eff} and VTR_{dual} to predict variations in GJ was diminished. The best-performing J_{eff} measure (red star in panel A) occurred at a cutoff value ρ_{cut} = 710 mgHA/cm^3 and was moderately correlated with GJ. B) VTR_{dual} achieved strong correlations with experimental GJ, including animals with widely varying healing responses.

Figure 6 – A) Idealized representation of secondary fracture healing with longitudinal changes in callus density and volume. B/C) Schematic time-history curves for J_{eff} and VTR in two fractures: one quick healer (grey) and one slow healer (blue). The quick healer progresses from soft callus (M) to hard callus (N) and finally remodeling and gap consolidation (O). In this example, the slow healer takes longer to achieve soft bridging (P), but eventually progresses through the same stages.

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