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Virtual Mechanical Tests Out-Perform Morphometric Measures for Assessment of Mechanical Stability of Fracture Healing *In Vivo*

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Abstract

Finite element analysis (FEA) with models derived from computed tomography (CT) scans is potentially powerful as a translational research tool because it can achieve what animal studies and cadaver biomechanics cannot – low-risk, non-invasive, objective assessment of outcomes in living humans who have actually experienced the injury or treatment being studied. The purpose of this study was to assess the validity of CT-based virtual mechanical testing with respect to physical biomechanical tests in a large animal model. Three different tibial osteotomy models were performed on 44 sheep. Data from 33 operated limbs and 20 intact limbs was retrospectively analyzed. Radiographic union scoring was performed on the operated limbs and physical torsional tests were performed on all limbs. Morphometric measures and finite element (FE) models were developed from CT scans and virtual torsional tests were performed to assess healing with four material assignment techniques. In correlation analysis, morphometric measures and radiographic scores were unreliable predictors of biomechanical rigidity, while the virtual torsion test results were strongly and significantly correlated with measured biomechanical test data, with high absolute agreement. Overall, the results validated the use of virtual mechanical testing as a reliable *in vivo* assessment of structural bone healing. This method is readily translatable to clinical evaluation for noninvasive assessment of the healing progress of fractures with minimal risk. Clinical significance: Virtual mechanical testing can be used to reliably and non-invasively assess the rigidity of a healing fracture using clinical-resolution CT scans and that this measure is superior to morphometric and radiographic measures.

Keywords: Finite element analysis; Computed tomography, Tibial shaft fracture; Ovine osteotomy;

Introduction

Finite element analysis (FEA) with models derived from computed tomography (CT) scans is increasing in prevalence as a research and clinical diagnostic tool in orthopaedics.[1–8] This technique is able to accurately capture both the complex geometries and localized material acquired by the CT scan to build representative animal-specific or patient-specific models.[9,10] FEA can then be used to simulate different loading scenarios and assess the structural biomechanics of the anatomy of interest.

With animal studies and cadaver biomechanics, structural outcomes can be directly measured. However, outcome assessments such as benchtop biomechanical testing and histology cannot be performed in clinical settings with human patients. Radiographic scoring and morphometric measures have been in use for decades to assess fracture healing but questions remain regarding reliability in large animal and clinical settings[11].Image-based models are potentially powerful as a clinically translatable research tool because they can achieve what animal studies and cadaver biomechanics cannot – low-risk, non-invasive, objective assessment of outcomes in living humans who have actually experienced the injury or treatment being studied. For example, we recently developed CT-derived computational methods to calculate the virtual torsional rigidity (VTR) of human tibial fractures after 12 weeks of healing.[9] In a pilot study, we showed that this measure is strongly correlated with clinical healing outcomes such as time to clinical union.[10] Furthermore, integrating image-based models in preclinical in vivo studies may reduce the required number of experimental animals, in accordance with 3R principles, by allowing non-invasive follow-up assessments of structural healing kinetics.

One barrier to widespread clinical use of patient-specific modeling techniques is that unlike in preclinical animal models, the quantitative predictions from these models cannot be easily validated due to a lack of physical biomechanical testing data in humans. Previous investigators have tried to bridge the gap between preclinical and clinical research techniques by using biomechanical testing of small animal osteotomy models to validate clinical assessments such as the radiographic union score (RUS or RUST when applied to the tibia). Recently, two independently conducted murine studies compared radiographic assessments with biomechanical data and CT-derived callus morphometry measures.[12,13] When interpreted together, these studies suggest that radiographic scoring is less reliable (lower inter-observer consistency) and less predictive of biomechanics (weaker correlation) at early timepoints in normal healing condition and with compromised fracture healing conditions compared to later timepoints in normal healing conditions. One previous study examined the repeatability of radiographic scoring when applied in well-healed ovine tibial osteotomies, but did not report correlations with the results of physical biomechanical testing.[14] These gaps in our ability to assess fracture union quantitatively across the spectrum of healing could be resolved by adoption of CT-based virtual biomechanics, but the necessary validation data showing that the virtual tests replicate the physical results have not yet been presented.

Accordingly, the purpose of this investigation was to assess the validity of CT-based virtual mechanical testing with respect to physical biomechanical testing in a large animal model. For this purpose, we used a series of standardized ovine (sheep) tibia osteotomies stabilized with internal fixation for which both CT scans and postmortem biomechanical testing data were available and which represented a wide spectrum of healing responses. The hypothesis of this

study was that the results of physical and virtual biomechanical testing would be strongly correlated.

Methods

Specimen Information:

Forty-four adult female Swiss alpine sheep (2-3 years old, weighing 59 – 87 kg) were part of two previously completed research studies with three different tibial osteotomy models (Figure 1) stabilized by medial plating. In total, there were 33 operated limbs and 20 intact control limbs that had both mechanical test data and CT scans available for analysis in this study. Taken together, these animals comprised three experimental datasets across a wide spectrum of healing responses. Dataset 1 consisted of data from seven animals with a 3 mm gap defect stabilized with a 12-hole stainless steel plate (broad straight veterinary 3.5 mm locking compression plate (LCP), 159 mm in length, with 3.5 mm bicortical screws; DePuy Synthes®). Dataset 2 consisted of data from 18 animals with a 3 mm gap defect stabilized with a six-hole titanium plate (broad 4.5/5.0 mm LCP, 115.8 mm in length, with 5 mm bicortical screws; DePuy Synthes®). Dataset 3 consisted of data from eight animals with a 17 mm defect augmented with autografts and stabilized with a 13-hole stainless steel plate (broad straight veterinary 3.5 mm LCP, 172 mm in length, with 3.5 mm bicortical screws; DePuy Synthes®). The 3 mm defect models (Datasets 1 and 2) represented a non-critical size defect capable of spontaneously healing. Sheep in Datasets 1 and 2 were sacrificed at 9 weeks. The 17 mm graft model (Dataset 3) represented a critical size defect that would not spontaneously heal without autograft augmentation. Sheep in Dataset 3 were sacrificed 12 weeks after surgery. All animal experiments were conducted according to the Swiss laws of animal protection and welfare and authorized by the local governmental veterinary authorities (license numbers ZH071/17 and ZH183/17).

Radiographic Union Scoring:

Plain film radiographs were taken postmortem at the time of sacrifice in three different planes: anteroposterior (AP), mediolateral (ML), and an angled plane between the AP and ML viewed from the cranial aspect of the limb. Radiographs were evaluated by two independent, board certified expert reviewers (Prof. Dr. med. vet. Brigitte von Rechenberg, Dipl. ECVS and Prof. Dr. med. vet. Mark Flückiger, Dipl. ECVDI). Semiquantitative radiographic union scores were assigned following a scoring system based in part on the modified radiographic union score for tibial fractures (mRUST) method.[15–17] Our scoring approach included the callus bridging assessment of clinical mRUST, except that due to medial plate fixation, only the lateral callus was scored from the AP view due to the presence of the medial plate. Half-point scores were allowed for greater granularity on the uniform osteotomies. The resulting unicortical score is hereafter referred to as lateral callus granular mRUST [score range 1 - 4 in 0.5 increments]. The callus bridging scores were performed on the AP radiograph. Additionally, the following scoring criteria we included for callus maturity, which are not typically assessed in clinical mRUST: cortical callus formation (AP projection) [0-4], cis-cortex callus formation (AP projection) [0-4], trans-cortex callus formation (AP projection) [0-4], cranial cortical gap (ML projection) [0-4], caudal cortical gap (angled projection) [0-4], and callus opacity scores (AP projection) [0-3]. All scoring components were summed, resulting in a comprehensive radiographic union score (cRUS) on an interval range [1-27] with higher scores representing more advanced healing. A scoring breakdown can be seen in the supplementary digital content.

Micro-Computed Tomography (μCT) Scanning:

After animal sacrifice, tibiae were excised, stripped of soft tissue, and all hardware was removed taking care not to disrupt the callus. Samples were then wrapped in saline-soaked

gauze, and μCT 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. A phantom (Scanco KP70 phantom, QRM, Moehrendorf, Germany) was scanned in the same scanner at identical settings allowing for conversion from native Hounsfield Units (HU) to calibrated mineral density (ρ_{QCT}, $mgHA/cm^3$).

Biomechanical Data

Physical torsion tests were performed on all included samples using a custom-made fixture on an Instron E10000 electro dynamic testing machine (Instron, Massachusetts, US). Axial loads and torques were measured with a calibrated load cell (±10 kN / ±100 N-m). Each tibia was prepared by stripping the periosteum on both ends and fixing the tibia in the test frame using Beracryl embedding medium. Periosteum was not stripped in the region of the callus, only at the embedded ends. To minimize bone movement in the Beracryl, four adjunctive screws were inserted perpendicular to the mechanical axis of the bone in the distal epiphyses. Potting depth was adjusted to ensure a minimal distance of 10 mm between the Beracryl and the nearest screw hole from the fracture plate. The spacing between the proximal and distal Beracryl surfaces after embedding was recorded and ranged between 140 mm and 160 mm. While potting, the diaphysis was kept moist using saline-soaked gauze. Mechanical tests were performed by quasi-statically preloading the limb with 5 N axially, which was held for the entire test, and then applying internal rotation at 5 °/min. Biomechanical torsional rigidity was calculated as a linear regression of the loading curve between 6 and 10 Nm multiplied by the gauge length, or the distance between the surfaces of the two Beracryl pots, of the test specimen during testing.

Scan processing and 3D model construction:

CT scans were processed using the Mimics Innovation Suite (Materialise, Leuven, Belgium) following a similar work flow as Schwarzenberg et al.[9] To better replicate the clinical scans used in the previous study and ensure future translatability, all of the μCT scans were initially down-sampled to an isotropic resolution of 400 μm prior to segmentation. Density threshold values of 400-2500 HU and 2500-4000 HU were chosen to initially segment the callus and cortical bone respectively (Figure 2c-d). Proximal and distal cut-planes were selected just interior to the most proximal and distal screw holes, resulting in two planar cortical surfaces for FE boundary condition (BC) application.

Following preliminary threshold-based segmentation of callus and cortical bone, all models were screened for formation of high-density tissue at the cortical-callus boundary, which can occur in samples with more advanced healing. To accomplish this, each callus mask was reviewed in a slice-by-slice manner throughout the CT-stack. In each slice, voxels of high-density new tissue that fell outside the contours of the original callus mask were assigned to a new tissue mask region. This step ensured that the callus morphometric measures were accurate and that the cortical bone fragments represented exclusively the original bone contour and none of the new tissue growth. After the mask for the new tissue was segmented, it was subtracted from the density-based cortical bone mask to create the true pre-existing cortical bone mask (Figure 2e-f). These regions are referred to as callus and bone within this work. For the callus region, volume and median density were recorded for each specimen. Final geometries were visually inspected by a surgeon to ensure the virtual models were anatomically representative of the biological samples. The final models for all operated limbs can be seen in Figure 3.

After the callus and bone masks were accurately segmented, the masks were united into a single surface model for preparation of volumetric discretization. The united models were

wrapped with a gap closing distance of 1 mm and a smallest detail of 0.5 mm to produce cohesive surfaces.

Finite element model creation:

The united surface models were exported to a dedicated toolkit in the Mimics Innovation Suite, 3-Matic, where the surface models were smoothed to further reduce CT scan noise and to fill any small defects. Next a triangular mesh was applied to the surface of each model with maximum edge lengths of 0.4 mm and a linear tetrahedral volumetric mesh was applied to the body of each model with maximum interior edge lengths of 0.875 mm. These parameters were selected based on a mesh convergence study where the virtual torsional rigidity changed by less than 1% from the previous step and to the next step.

Elementwise material properties were initially applied to the FE meshes (Figure 2h) by three non-species-specific methods: one linear and two power law material assignments taken from the literature (Figure 4). First, a direct conversion from HU to elastic modulus was achieved using a linear scaling law.[18] For the two power law material assignments, the native HU density measure of the CT scan was first converted to calibrated mineral density (ρ_{QCT}) using the phantom and then to ash $\left(\frac{\rho_{QCT}+90}{1.14}\frac{mg}{cm^3}\right)$ and apparent density $\left(1.54\times\rho_{ash}+150\,\frac{mg}{cm^3}\right)$ using equations from Schileo et al.[19] Once voxel density values were in ash and apparent densities, material scaling law equations were used from Keyak et al.[20] $\left(10.5\times\frac{\rho_{ash}}{1000}^{2.57}\,GPa\right)$ and Morgan et al.[21] $\left(8.92\times\frac{\rho_{app}}{1000}^{1.83}\,GPa\right)$ respectively to calculate elastic modulus. These two power law equations were chosen to represent the lower and higher end of elastic modulus assignment from

two literature reviews.[22,23] A species-specific scaling law for ovine tibial cortical bone was not identified from a literature search and was subsequently developed in this study.

Virtual torsion testing

In preclinical studies, torsion testing is the preferred mode of assessment due to its robustness to variation in setup orientation. Structural FE simulations replicating this torsional test were carried out in ANSYS 17.2 (Canonsburg, Pennsylvania). The boundary conditions were rigid fixation on the distal end and 1 degree of applied rotation on the proximal end, with twisting about the aligned mechanical axis of the tibia. Additionally, using the same multi-point constraint (MPC) contact of the applied axial rotation, the proximal end was fixed in the anterior-posterior and medial-lateral translational directions to best replicate the physical test setup (Figure 2i). For each model, the virtual torsional rigidity (VTR) was calculated as the moment reaction from the applied loading (M) multiplied by the working length of the test segment (L) divided by the applied angle of twist (ϕ): $VTR = ML/\phi$.

Material Optimization

An optimization technique similar to Eberle et al.[24] was performed on the N = 20 intact tibiae to find a material assignment law of the form $E = a\rho_{QCT}^{b}$ that best fit the data. In brief, value ranges for a ($5{,}000 \leq a \leq 20{,}000$) and b ($1 \leq b \leq 3$) were chosen from the same literature review as above[22,23] to represent the current published data across multiple anatomic sites. Sixteen design points were chosen to span the parameter space and the torsional rigidity of each intact model was calculated by assigning all elementwise material properties within all N = 20 models using the coefficients a and b at those points. A root mean square error (RMSE) was calculated comparing each design point (coefficients a and b) to the biomechanical data and a 2nd

order response surface was fit to the *a*, *b*, and RMSE values. The minimum predicted RMSE of the response surface was calculated by 120,801 sample points in the design space, defined by a resolution of 25 $MPa\frac{cm^3}{gHA}$ in the *a* dimension and 0.01 in the *b* dimension. After the initial response surface minimum was calculated, three additional refinement design points were iteratively computed from the surface minimum. Finally, the minimum *a* and *b* values for the response surface with 19 design points (16 preliminary and 3 refinement) was used to assign elementwise material properties in all N = 33 operated limb models and N = 20 intact limb models. The resulting virtual torsional rigidities (VTRs) were calculated for further statistical analysis.

Statistical Analysis

Descriptive statistics were generated using Microsoft Excel and MATLAB (R2016a-R2019a, The MathWorks, Inc., Natick, Massachusetts). Additional statistical analyses were conducted in SPSS 25 (IBM Corp., Armonk, NY). Pearson correlations were performed to assess the strength of the linear association between various outcome measures and the measured biomechanical rigidity from physical testing. Correlation coefficients were interpreted as follows[25,26]: weak $R^2 \leq 0.3$, moderate $0.3 < R^2 < 0.6$, or strong $R^2 \geq 0.6$. One-way repeated-measures ANOVAs were computed to determine group differences between the biomechanical rigidity and the simulated VTR data. Additionally, root mean squared error (RMSE) analyses were performed to evaluate the relative performance of the four material property assignment techniques (three non-species-specific, one ovine-optimized). All reported values are medians and interquartile ranges (IQR) unless otherwise reported and statistical significance was at $p < 0.05$.

Results

Structural Data: Biomechanical Testing

The results of physical biomechanical testing for all samples are shown in Figure 5 and Table 1. Dataset 3 (17 mm defect with graft) had the lowest torsional rigidity, as expected in this delayed healing model. Between-groups comparisons were not performed between Datasets 1, 2 and 3 because these experiments were not conducted concurrently with intent to compare outcomes. Instead, these torsional rigidity results were combined into a single osteotomy dataset, representing a range of healing responses, and were subsequently used as a benchmark to assessing the reliability of various outcomes measures (morphometric, radiographic, and virtual biomechanics) with respect to the measured physical properties of the specimens.

Morphometric Data

Summary morphometric data for the three osteotomy datasets individually and for all samples together is presented in Table 2. Callus volume was weakly-to-moderately and significantly correlated with biomechanical torsional rigidity in Datasets 2 and 3 and for the 33 combined osteotomies overall. Callus density was weakly and non-significantly associated with biomechanical torsional rigidity in all datasets and for the 33 combined osteotomies overall.

Radiographic Data

Radiographic scoring data can be seen in Table 3. The lateral callus granular mRUST score was moderately and significantly correlated with biomechanical torsional rigidity in Dataset 1 only, but weakly and non-significantly correlated with the other two datasets and the 33 combined osteotomies overall. Similarly, the comprehensive radiographic union score (cRUS) was strongly and significantly correlated with biomechanical torsional rigidity in Dataset 1 only,

with weak and non-significant correlations for the other two datasets and the 33 combined osteotomies overall.

Structural Data: Virtual Torsional Rigidity (VTR)

Virtual torsional rigidity (VTR) data from all simulations is shown in Figure 5 and Table 4. As described above, each of the N = 33 osteotomy limbs and N = 20 intact limbs were subjected to virtual testing with four different material assignment approaches: three non-species-specific scaling laws (Synder linear, Morgan power law, and Keyak power law) and one optimized species-specific ovine power law. Across all osteotomy datasets, the combined osteotomies, and the intact limbs, regardless of material assignment law, the virtual and physical biomechanical tests were moderately-to-strongly and significantly correlated. Unsurprisingly, the VTRs resulting from the optimized ovine-specific material law performed as well or better than the non-optimized non-species-specific material assignment approaches.

Figure 5 compares the predicted VTR values for the combined osteotomies and the intact limbs to the physical biomechanical torsional rigidity. Significant differences are indicated with respect to physical biomechanical torsional rigidity. The non-species-specific material assignment laws significantly over-predicted rigidity, despite their high correlation with the biomechanical test results. The RMSE analysis showed that in both the fractured and intact models, the Keyak power law material assignment method had the lowest relative error of the non-species-specific material assignment laws (Table 5).

Discussion

The goal in many preclinical orthopaedic studies is to measure the healing progress of the fractured bone with a surrogate measure such as torsional stiffness or rigidity. To accomplish

this, animals need to be sacrificed and bones excised for physical testing, which cannot be done in clinical studies or at interim timepoints in an animal model. Currently, morphometric measurement of callus formation (volume and density) is a commonly used technique that can be performed *in vivo* to evaluate fracture healing progress prior to animal sacrifice.[27,28] Furthermore, the clinical gold-standard radiographic scoring systems such as RUST and mRUST are used in both preclinical and clinical settings to track healing progress. In this study, our method of virtual torsion testing outperformed all of these measures when predicting the biomechanical rigidity of the bone. Virtual torsion testing with the species-specific material model also produced strong and statistically significant correlations and low error between the measured and simulated biomechanical properties across a wide range of healing responses.

The study results also indicate that morphometric measures of callus formation should be interpreted with caution. Callus volume and density are often considered surrogate measures of healing progress or even indirect indicators of biomechanical properties. In this context, callus volume is analogous to the amount of new tissue visible on plain film radiographs, while callus density can help infer tissue maturity and structural integrity. Both are interpreted as signs of healing.[29] In this study, callus volume had a moderate correlation with measured torsional rigidity in Dataset 1 and Dataset 3 and weak correlations with torsional rigidity in Dataset 2. Dataset 3 was designed to represent a critical size defect which is more indicative of a delayed healing case which may have more dependency on callus volume than a non-critical size defect model. Furthermore, this dependency on callus volume may be attributed to Dataset 3 being a delayed healing model in which new bone is still expected to significantly remodel and condense after the analyzed endpoint of 12 weeks. However, within all datasets, callus density showed little association with biomechanics, which could be because all callus present was of similar

maturity. In a preclinical model with more widely varying timepoints, callus density could be a more important indicator of healing progress. In our controlled osteotomy models the coefficients of variation for callus volume (36%, 38% and 24%) were much higher than for callus density (10%, 8% and 7%). This is consistent with callus volume and density patterns observed in humans at 12 weeks[10]. Notably, the plates used for fixation were not the same across datasets, which could explain some of the differences in healing response, although as previously stated, between-groups comparison of the fixators was not the objective of this investigation. In fact, by design we aimed to show the validity of our hypothesis across various fixation techniques and defect sizes. The observed low correlation of morphometric measures with outcome parameters seen in this study are consistent with findings from our clinical pilot studies, which used the same virtual mechanical testing technique and showed that morphometric parameters are not strongly correlated with simulated VTR or with clinical outcomes such as time to union.[10] It is important to acknowledge that in some study designs, callus volume and density may still be import measures of the healing process and could be included as complementary findings together with virtual mechanical tests.

Additionally, the radiographic scoring results do not provide a consistent picture. The radiographic scores in Dataset 1 had much stronger correlations with biomechanical rigidity than the other two models. The callus in Dataset 1 also tended to be the largest (Table 2). This could suggest that visual radiographic scoring is more reliable when there is more callus present. If true, this would be consistent with murine data showing that radiographic scoring is less reliable and less predictive of biomechanics at early timepoints and with compromised fracture healing compared with later timepoints in normal healing conditions[11].

Overall, this study validated the robustness of virtual mechanical testing as an *in vivo* method for assessing structural bone healing. With the optimized species-specific material assignment law for ovine tibial cortical bone, we found a strong and statistically significant correlation between virtual and physical torsional rigidity across all sample subgroups, which was superior to both radiographic and morphometric assessments from CT. Even with non-species-specific (human) material models, the virtual mechanical test results were still moderately-to-strongly and significantly correlated with physical biomechanical testing. This finding is particularly notable because it indicates that virtual mechanical testing may be a powerful *in vivo* assessment tool, even when a robust species- and site-specific material assignment law is not available.

This study had a few additional minor limitations that may be restricting the predictive power and correlations between measures. First, as in all physical biomechanical torsion tests, there were small potting artifacts where the bone was able to slightly deform within the PMMA pot. This could reduce the measured stiffness and rigidity of the biomechanical data, raising the relative error between physical and simulated data. Furthermore, this study only investigated the most common loading scenario in preclinical studies, a torsion test, not other modes of loading. While torsional tests are the most common preclinical measurements, it may not be the best representation of load bearing capacity which also include axial and bending components[30,31]. Additionally, while higher resolution μCT scans were acquired, the scans were down sampled to replicate the resolution of clinical scanners. Starting with a less resolute data set eliminates the possibility of more sophisticated structural material modeling, such as the use of fabric tensors, and could have also introduced mask boundary definition errors in the virtual models. However, μCT scan data is not available in a clinical setting or at interim *in vivo* timepoints in large animal models, so the clinical resolution chosen here has clear translational relevance.

Finally, it is important to note that the optimized scaling law we developed for the ovine tibial diaphysis using the intact limbs may not accurately model the properties of callus tissue at all stages of maturity. The techniques we have describe can only be implemented when mineralized callus become radio-opaque. At the timepoint our scans were taken, fracture callus consisted of fibrous tissue, some cartilage, and woven bone, which is not as organized or mineralized as cortical bone. During coupled remodeling, callus microstructure, composition, and mechanics change adaptively[32], and the modeling approach we are using my not be adequate to capture all of those processes. Optimization of new material property scaling laws for combined cortical-callus structures should be a target for continuing research and would undoubtedly reduce the RMSE of the virtual prediction.

Conclusions

Image-based structural mechanics modeling from CT scans is an objective, quantitative method for assessing fracture healing that can closely replicate physical biomechanical testing. Using the methods described herein, we validated a technique for virtual torsional testing using ovine tibial osteotomy fracture models representing a wide range of healing scenarios. The virtual mechanical testing results were better predictors of trends in measured biomechanical properties than radiographic scoring or morphometric analysis of callus from CT scans in these models. This method is readily translatable to preclinical or clinical settings for noninvasive assessment of the structural healing progress of fractured long bones with minimal risk.

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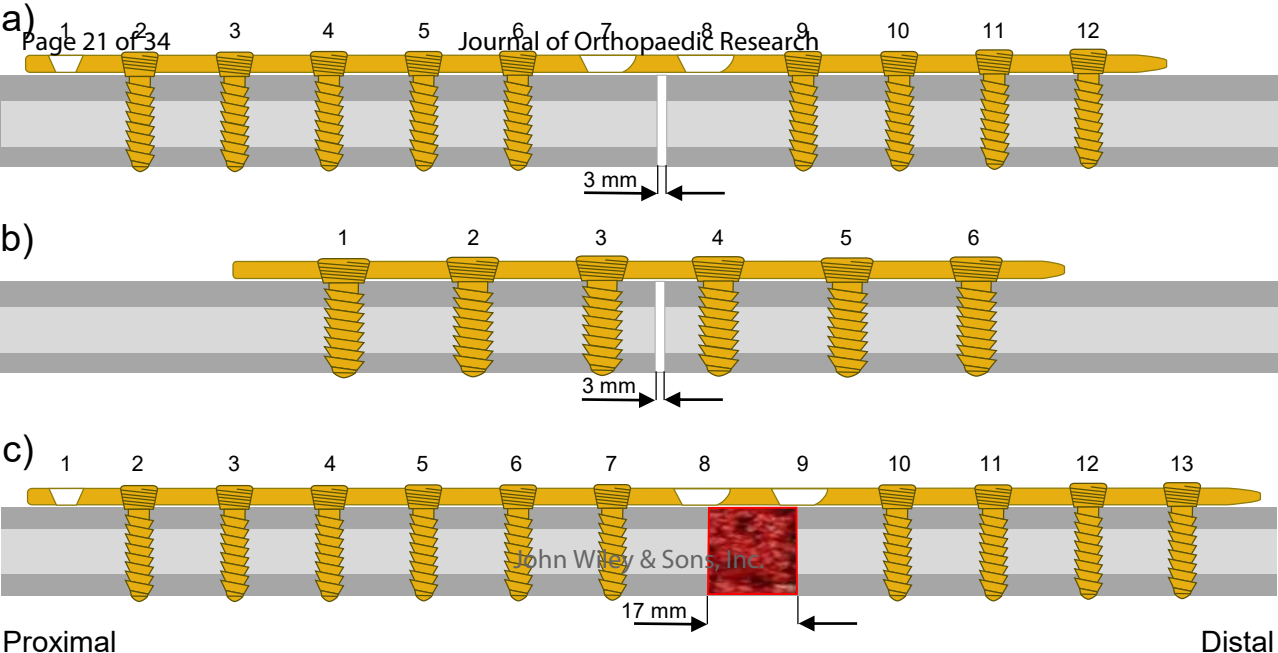
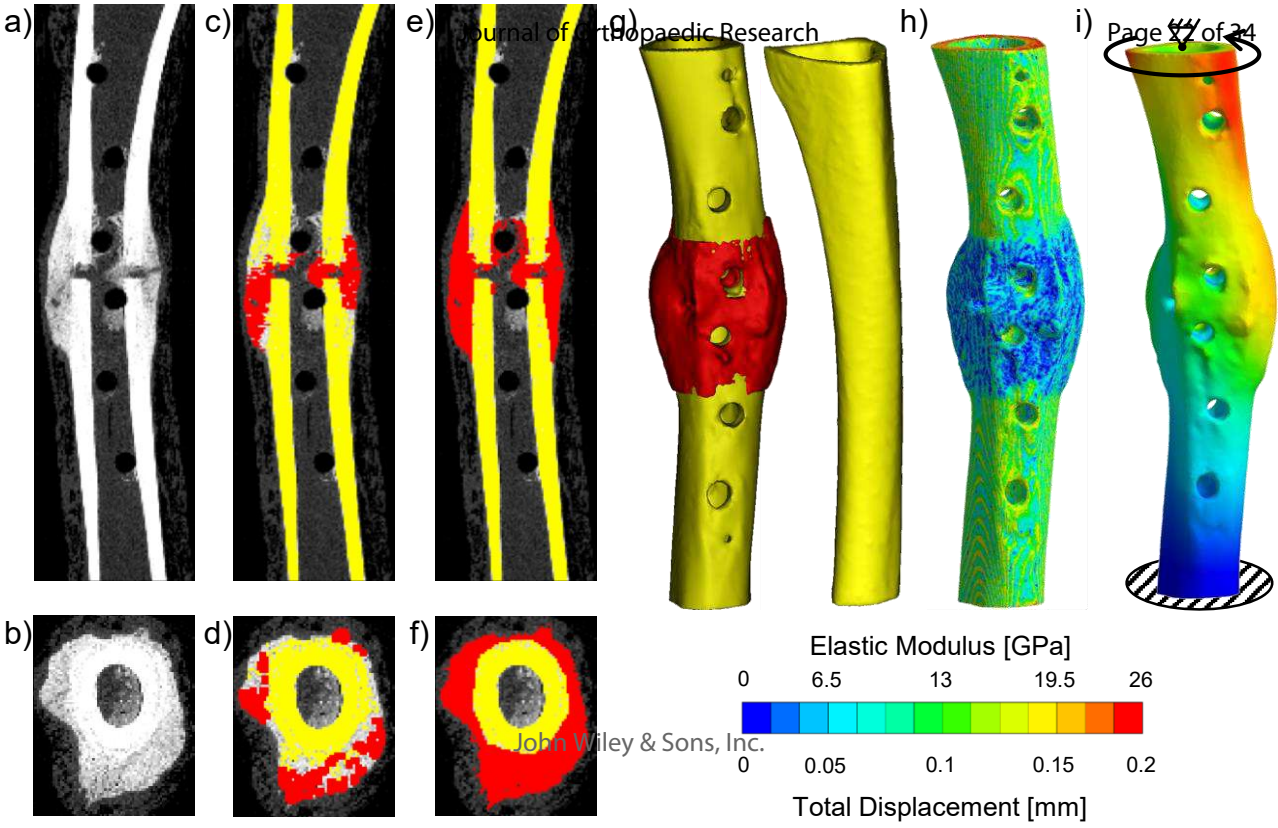
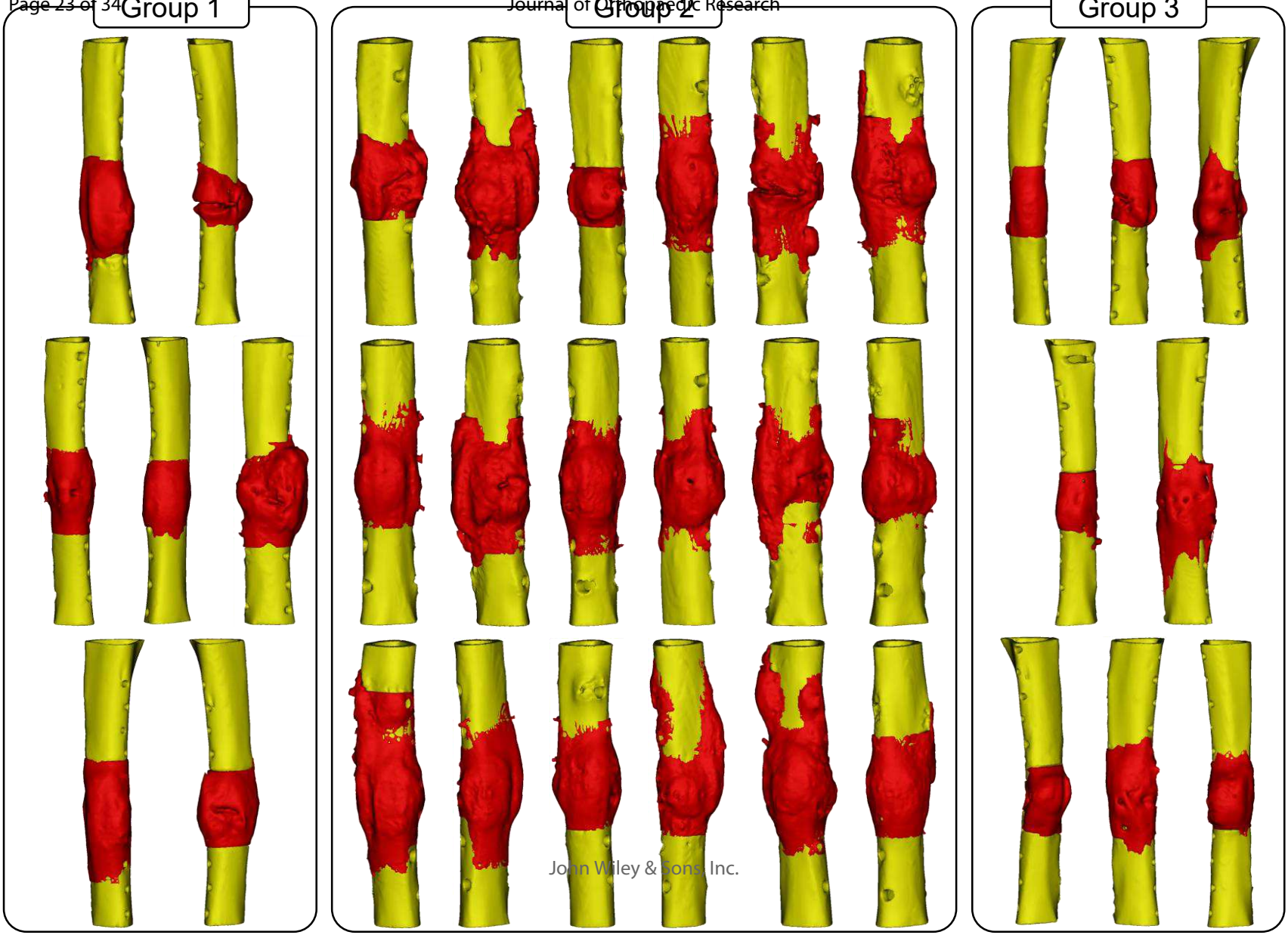
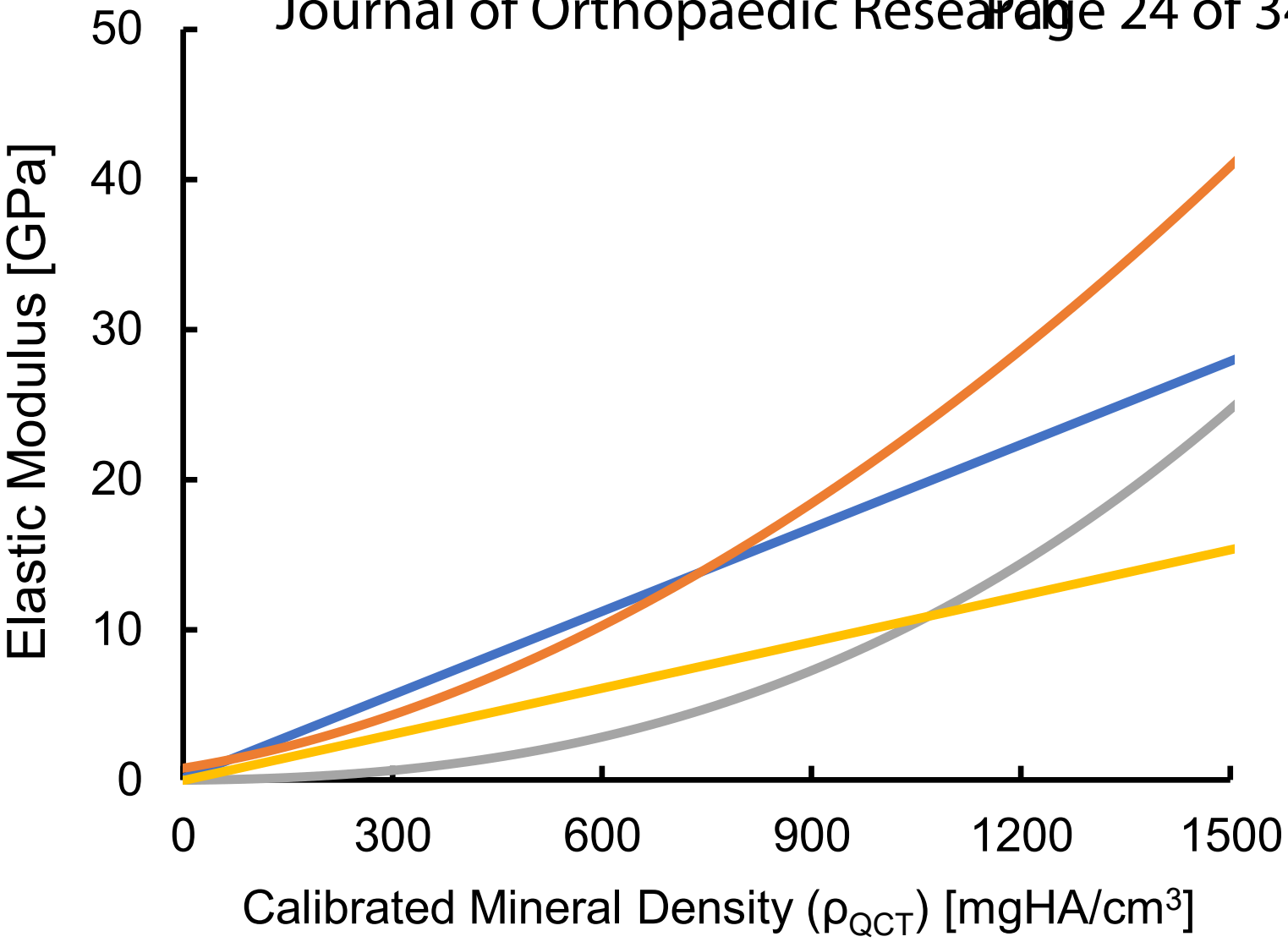


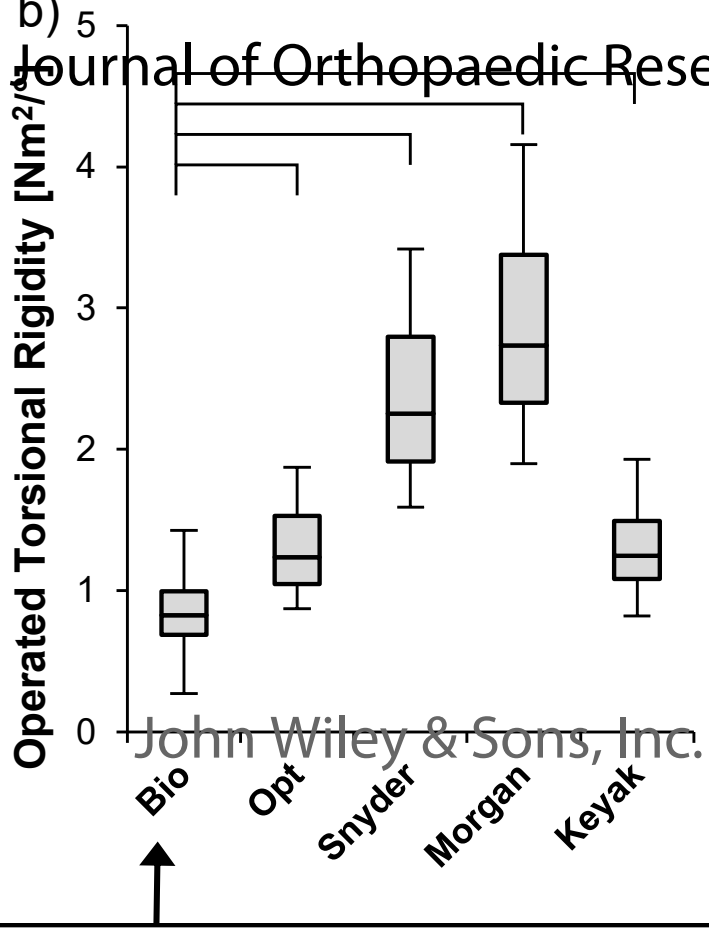


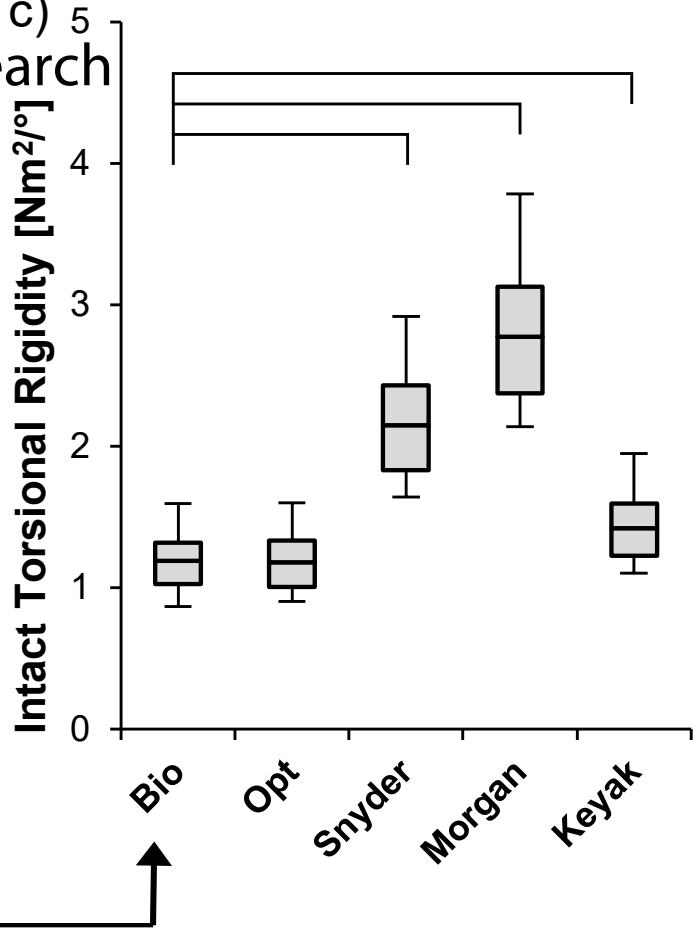


Elastic Modulus vs Radiodensity



Snyder 1991	E = 0.00704×HU
Morgan 2003	$E = 8.92 \times \rho_{app}^{1.83}$
Keyak 1994	$E = 10.5 \times \rho_{ash}^{2.57}$
Optimized	$E = 10.23 \times \rho_{QCT}$





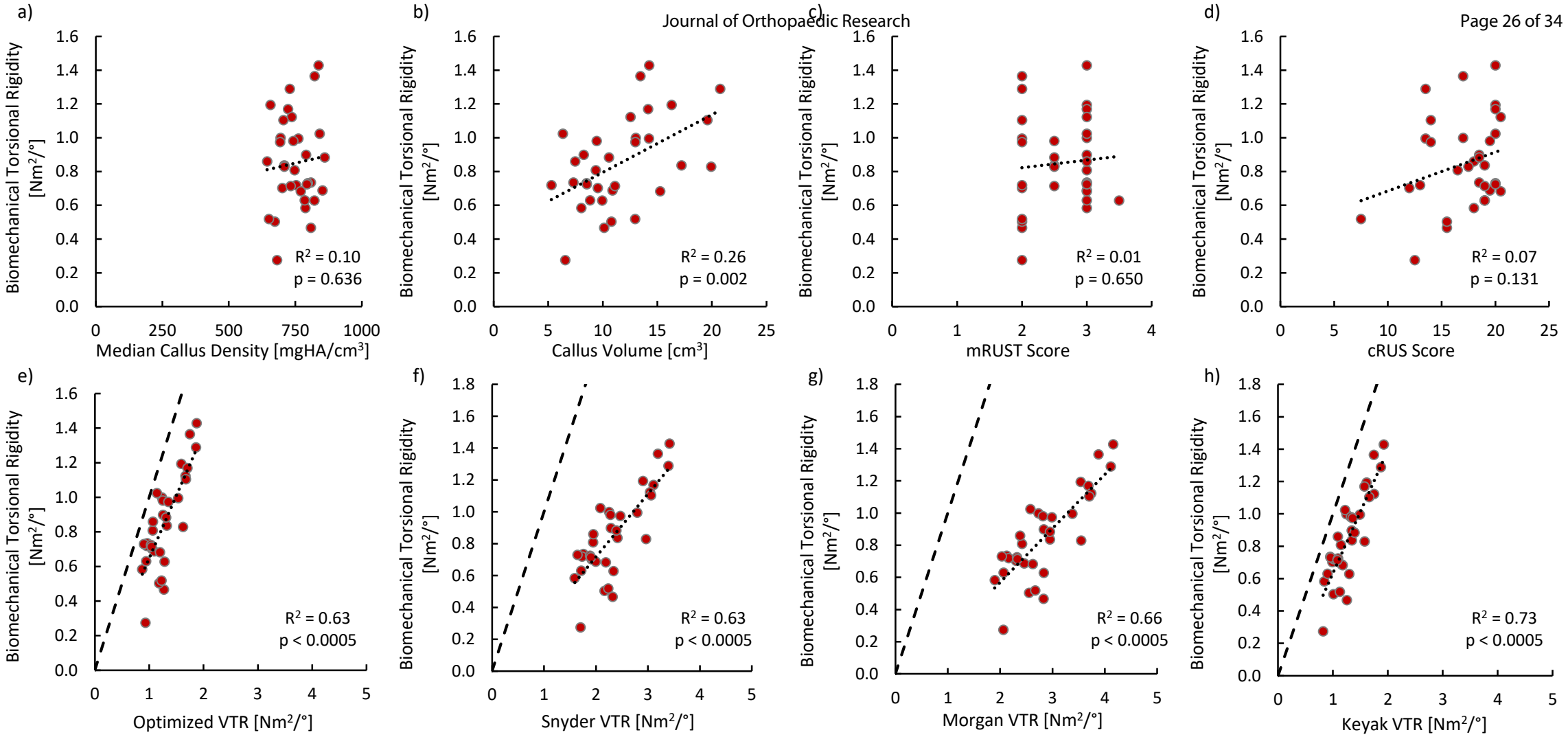

a)
Biomechanical Torsional Rigidity [Nm²/°]
Median Callus Density [mgHA/cm³]
R² = 0.10
p = 0.636
b)
Biomechanical Torsional Rigidity [Nm²/°]
Callus Volume [cm³]
R² = 0.26
p = 0.002
c)
Biomechanical Torsional Rigidity [Nm²/°]
mRUST Score
R² = 0.01
p = 0.650
d)
Biomechanical Torsional Rigidity [Nm²/°]
cRUS Score
R² = 0.07
p = 0.131
e)
Biomechanical Torsional Rigidity [Nm²/°]
Optimized VTR [Nm²/°]
R² = 0.63
p < 0.0005
f)
Biomechanical Torsional Rigidity [Nm²/°]
Snyder VTR [Nm²/°]
R² = 0.63
p < 0.0005
g)
Biomechanical Torsional Rigidity [Nm²/°]
Morgan VTR [Nm²/°]
R² = 0.66
p < 0.0005
h)
Biomechanical Torsional Rigidity [Nm²/°]
Keyak VTR [Nm²/°]
R² = 0.73
p < 0.0005

Table 1: Biomechanical torsional rigidities for each osteotomy group, all osteotomies combined, and the intact tibiae.

		Measured Torsional Rigidity [Nm²/°]	
Dataset	**Sample Size**	**Median**	**IQR**
Dataset 1	7	1.02	(0.79 - 1.16)
Dataset 2	18	0.82	(0.71 - 0.89)
Dataset 3	8	0.72	(0.60 - 1.04)
All Osteotomized Tibiae	33	0.83	(0.69 - 1.00)
All Intact Tibiae	20	1.19	(1.03 - 1.32)

Table 2: Callus morphometric data and correlations to biomechanical torsional rigidity for each group.

	Measure	Median	IQR	R^2 with Biomech data	*p* with Biomech data
Dataset 1	Callus Volume [cm^3]	12.54	(7.30 - 13.63)	0.56	0.055
	Callus Density [$mgHA/cm^3$]	736	(688 - 813)	0.09	0.516
Dataset 2	Callus Volume [cm^3]	10.73	(8.75 - 14.69)	0.23	**0.044**
	Callus Density [$mgHA/cm^3$]	745	(709 - 793)	0.03	0.475
Dataset 3	Callus Volume [cm^3]	10.46	(9.36 - 13.63)	0.53	**0.040**
	Callus Density [$mgHA/cm^3$]	774	(717 - 809)	0.06	0.568
All Osteotomized Tibiae	Callus Volume [cm^3]	10.79	(8.54 - 14.16)	0.26	**0.002**
	Callus Density [$mgHA/cm^3$]	748	(705 - 797)	0.01	0.636

Table 3: Radiographic scores and correlations to biomechanical torsional rigidity for each group.

	Measure	Median	IQR	R^2 with Biomech data	*p* with Biomech data
Dataset 1	Lateral Callus Granular mRUST	3.0	(3.0 - 3.0)	0.58	**0.048**
	Total Radiographic Score (cRUS)	20.0	(17.5 - 20.0)	0.72	**0.016**
Dataset 2	Lateral Callus Granular mRUST	2.5	(2.13 - 3.0)	0.18	0.075
	Total Radiographic Score (cRUS)	18.5	(14.6 - 19.4)	0.01	0.714
Dataset 3	Lateral Callus Granular mRUST	2.0	(2.0 - 3.0)	0.00	0.888
	Total Radiographic Score (cRUS)	16.3	(15.0 - 18.6)	0.06	0.566
All Osteotomized Tibiae	Lateral Callus Granular mRUST	3.0	(2.0 - 3.0)	0.01	0.650
	Total Radiographic Score (cRUS)	18.5	(15.5 - 19.5)	0.07	0.131

Table 4: Virtual Torsional Rigidities (VTRs) and correlations to biomechanical torsional rigidity for each group.

	Resultant Virtual Torsional Rigidity (VTR) with Each Material Assignment Law	Median	IQR	Unit	R with Biomech data	R^2 with Biomech data	*p* with Biomech data
Dataset 1	VTR - Optimized	0.94	(0.70 - 1.20)	Nm2/°	0.89	0.79	**0.008**
	VTR - Snyder	2.34	(1.78 - 2.99)	Nm^2/°	0.89	0.78	**0.008**
	VTR - Morgan	2.83	(2.14 - 3.67)	Nm^2/°	0.89	0.79	**0.008**
	VTR - Keyak	1.29	(0.87 - 1.56)	Nm^2/°	0.92	0.85	**0.003**
Dataset 2	VTR - Optimized	0.86	(0.81 - 0.96)	Nm2/°	0.71	0.50	**0.001**
	VTR - Snyder	2.14	(2.04 - 2.43)	Nm^2/°	0.71	0.50	**0.001**
	VTR - Morgan	2.61	(2.50 - 2.94)	Nm^2/°	0.74	0.54	**0.001**
	VTR - Keyak	1.24	(1.14 - 1.35)	Nm^2/°	0.79	0.63	**< 0.0005**
Dataset 3	VTR - Optimized	0.83	(0.75 - 1.18)	Nm2/°	0.82	0.67	**0.013**
	VTR - Snyder	2.09	(1.88 - 2.94)	Nm^2/°	0.82	0.67	**0.013**
	VTR - Morgan	2.5	(2.26- 3.55)	Nm^2/°	0.82	0.67	**0.012**
	VTR - Keyak	1.08	(0.95 - 1.57)	Nm^2/°	0.84	0.71	**0.008**
All Osteotomized Tibiae	VTR - Optimized	0.87	(0.78 - 1.14)	Nm2/°	0.80	0.63	**< 0.0005**
	VTR - Snyder	2.15	(1.95 - 2.84)	Nm^2/°	0.80	0.63	**< 0.0005**
	VTR - Morgan	2.65	(2.35 - 3.44)	Nm^2/°	0.81	0.66	**< 0.0005**
	VTR - Keyak	1.22	(1.05 - 1.54)	Nm^2/°	0.86	0.73	**< 0.0005**
All Intact Tibiae	VTR - Optimized	0.81	(0.70 - 0.93)	Nm2/°	0.84	0.70	**< 0.0005**
	VTR - Snyder	2.00	(1.73 - 2.29)	Nm^2/°	0.84	0.70	**< 0.0005**
	VTR - Morgan	2.60	(2.23 - 2.97)	Nm^2/°	0.84	0.71	**< 0.0005**
	VTR - Keyak	1.34	(1.14 - 1.53)	Nm^2/°	0.85	0.73	**< 0.0005**

Table 5: Root mean squared error with biomechanical torsional rigidity for each group.

	Structural Model	RMSE with Biomechanical Rigidity [Nm²/°]
Dataset 1	Optimized	0.42
	Snyder	1.53
	Morgan	2.08
	Keyak	0.43
Dataset 2	Optimized	0.46
	Snyder	1.50
	Morgan	2.00
	Keyak	0.48
Dataset 3	Optimized	0.51
	Snyder	1.59
	Morgan	2.08
	Keyak	0.45
All Osteotomized Tibiae	Optimized	0.47
	Snyder	1.53
	Morgan	2.04
	Keyak	0.46
All Intact Tibiae	Optimized	0.11
	Snyder	0.99
	Morgan	1.63
	Keyak	0.27

ARRIVE

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK, [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Title
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Abstract
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Supplementary Information
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Introduction
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Methods/Specimen Info
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	Supplementary Information
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	Methods and Supplementary Information
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	Methods/Specimen Information and Supplementary Information

The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010[1]

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	Supplementary Information
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	Methods and Supplementary Information
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	Supplementary Information
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Methods
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	Methods/Statistical Analysis
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	Supplementary Information
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	Methods/Specimen Information and Supplementary Information
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Results
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	Supplementary Information
DISCUSSION			
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	Results and Discussion
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Discussion and Conclusion
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	Acknowledgements

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.