



# Is there a Difference in Interfragmentary Compression Strength Between Fully or Partially Threaded Screws? Results of an Experimental **Biomechanical Pilot Study**

Há diferença na força de compressão interfragmentar entre parafusos com rosca total ou parcial? Resultados de um estudo biomecânico experimental piloto

Tosan Okoro<sup>1</sup> Marcus Landgren<sup>2,3</sup> Edem Afenu<sup>4</sup> Gabriele Russow<sup>5,6</sup> Dag Wulsten<sup>6</sup> Mark Heyland<sup>6</sup>

- <sup>1</sup>Department of Arthroplasty, Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust, Gobowen, Oswestry SY10 7AG, United Kingdom
- <sup>2</sup> Department of Orthopedic Surgery, Hand Surgery Unit, Copenhagen University Hospital - Herlev and Gentofte, Gentofte, Denmark
- <sup>3</sup>Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
- <sup>4</sup>University of Toronto Faculty of Medicine, 1 Kings College Circle, Toronto M4Y 2V6, Ontario, Canada
- <sup>5</sup>Charité Universitätsmedizin Berlin, Centre for Musculoskeletal Surgery, Berlin, Germany
- <sup>6</sup>Berlin Institute of Health at Charité Universitätsmedizin Berlin, Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Berlin, Germany

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Address for correspondence Mark Heyland, Doctor of Medicine, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Julius Wolff Institut, Berlin, Germany (e-mail: mark.heyland@bih-charite.de).

# Abstract

#### **Keywords**

- biomechanical phenomena
- bone cements
- bone screws
- ► fractures, bone

**Objective** This study assessed differences between fully- and partially-threaded screws in the initial interfragmentary compression strength. Our hypothesis was that there would be an increased loss in initial compression strength with the partially-threaded screw.

**Methods** A 45-degree oblique fracture line was created in artificial bone samples. The first group (FULL, n=6) was fixed using a 3.5-mm fully-threaded lag screw, while the second group (PARTIAL, n=6) used a 3.5-mm partially-threaded lag screw. Torsional stiffness for both rotational directions were evaluated. The groups were compared based on biomechanical parameters: angle-moment-stiffness, time-moment-stiffness, maximal torsional moment (failure load), and calibrated compression force based on pressure sensor measurement.

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Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Berlin, Germany.

Results After loss of one PARTIAL sample, no statistically significant differences in calibrated compression force measurement were observed between both groups: [median (interquartile range)] FULL: 112.6 (10.5) N versus PARTIAL: 106.9 (7.1) N, Mann-Whitney U-test: p = 0.8). In addition, after exclusion of 3 samples for mechanical testing (FULL n=5, PARTIAL n=4), no statistically significant differences were observed between FULL and PARTIAL constructs in angle-moment-stiffness, timemoment-stiffness, nor maximum torsional moment (failure load).

**Conclusion** There is no apparent difference in the initial compression strength (compression force or construct stiffness or failure load) achieved using either fullyor partially-threaded screws in this biomechanical model in high-density artificial bone. Fully-threaded screws could, therefore, be more useful in diaphyseal fracture treatment. Further research on the impact in softer osteoporotic, or metaphyseal bone models, and to evaluate the clinical significance is required.

# Resumo

Objetivo Este estudo avaliou diferenças entre parafusos com rosca total ou parcial na resistência à compressão interfragmentar inicial. Nossa hipótese era de que haveria maior perda de resistência à compressão inicial com o parafuso de rosca parcial.

Métodos Uma linha de fratura oblíqua de 45 graus foi criada em amostras de osso artificial. O primeiro grupo (TOTAL, n = 6) foi fixado com um parafuso de 3,5 mm de rosca total, enquanto o segundo grupo (PARCIAL, n = 6) usou um parafuso de 3,5 mm de rosca parcial. Avaliamos a rigidez à torção em ambas as direções de rotação. Os grupos foram comparados com base nos seguintes parâmetros biomecânicos: momento de rigidez-ângulo, momento de rigidez-tempo, momento de torção máxima (carga de falha) e força de compressão calibrada com base na medida do sensor de pressão.

Resultados Depois da perda de uma amostra PARCIAL, não foram observadas diferenças estatisticamente significativas na força de compressão calibrada entre os 2 grupos [mediana (intervalo interquartil)]: TOTAL: 112,6 (10,5) N e PARCIAL: 106,9 (7,1) N, com p = 0.8 segundo o teste U de Mann-Whitney). Além disso, após a exclusão de 3 amostras para testes mecânicos (TOTAL, n=5, PARCIAL, n=4), não foram observadas diferenças estatisticamente significativas entre os construtos TOTAL e PARCIAL quanto ao momento de rigidez-ânqulo, momento de rigidez-tempo ou momento de torção máxima (carga de falha).

Conclusão Não há diferença aparente na força de compressão inicial (força de compressão ou rigidez do construto ou carga de falha) com o uso de parafusos de rosca total ou parcial neste modelo biomecânico em osso artificial de alta densidade. Parafusos de rosca total podem, portanto, ser mais úteis no tratamento de fraturas diafisárias. Mais pesquisas são necessárias sobre o impacto em modelos ósseos osteoporóticos ou metafisários de menor densidade e avaliação do significado clínico.

# **Palavras-chave**

- ► fenômenos biomecânicos
- cimentos ósseos
- ▶ parafusos ósseos
- ► fraturas ósseas

# Introduction

Orthopedic screws have been improved in terms of material composition, thread count, shape, pitch, and diameter to allow optimization for a variety of bone types, qualities, and pathologies.<sup>1</sup> Partially-threaded screws are generally smooth from the screw head to half or two thirds of their length, while fully-threaded screws have threads that run throughout their entire length.<sup>2</sup> Lag screw fixation involves the placement of partially or fully-threaded bone screws in a direction perpendicular to the plane of the fracture site to achieve interfragmentary compression between bone fracture fragments to ensure bone fracture healing.<sup>3</sup> To achieve compression, over-drilling of the near cortex or use of a partially-threaded screw has been taught, 4 so that the far (trans-)fragment can be pulled toward the near (cis-) fragment.

The lag screw technique is well utilized with spiral or oblique fractures, as it restores the premorbid anatomic alignment of the bone but also provides stable

interfragmentary compression between fragments. It is used to help resolve olecranon, mandibular, malleolar, sacral, tibial, and femoral fractures, among others. 5-10 A study comparing fully-threaded cancellous lag screws to partially-threaded cancellous screws on the basis of shear, stiffness, and yield load by using saw-bone blocks to apply axial compression on the screws found that fully-threaded screws were biomechanically superior with regards to initial stiffness and failure load.<sup>2</sup> Another study also aimed to determine differences between fully-threaded and partiallythreaded cancellous screws based on their stability by using a porcine model that simulated slipped capital femoral epiphysis (SCFE). The authors found no significant differences between fully-threaded or partially-threaded cancellous screws comparing loads per displacement, indicating the suitability of both types of screws for use in treating fractures. 11 The vast clinical similarities between fully and partially-threaded lag screws is also highlighted in a human study that aimed to evaluate medial malleolar fractures which were treated with either fully-threaded headless compression screws or partially-threaded screws using clinical and radiological prognostic criteria. This study suggests satisfactory results regarding fixation technique for either screw type. 12

Little is known about the initial compression strength with either fully- or partially-threaded screws. We assume the fully-threaded screw in an over-drilled hole will show superior strength as we do not believe the sliding in the cis cortex that existed during the creation of compression will prevail during loading, and the fully-threaded screw will provide more rigidity due to a locking of the screw threads.

The purpose of this biomechanical pilot study is to assess differences between fully- and partially-threaded screws in the initial interfragmentary compression strength (failure load in torsion) and in the construct stiffness under load. Our hypothesis is that there is a detectable loss of initial compression strength with the partially-threaded screw, as well as lower stiffness, as there is no screw thread contact at the nearby (cis-)fragment.

## **Methods**

Artificial bone samples (hard composite fiber tube, density: 1.1–1.4 g/cm<sup>3</sup>, outer diameter 30 mm, inner diameter 20 mm, total n = 12, 6 per group,  $ightharpoonup Fig. 1)^{13,14}$  were equally separated into two groups: The first (FULL) and second (PARTIAL) groups received 45-degree oblique fractures using an oscillating saw. The nearby (cis-)fragment was drilled with a 4-mm drill bit (overdrilled) and the far (trans-)fragment was drilled with a 2.5-mm drill bit perpendicular to the oblique fracture line for all samples. The 3.5-mm fully threaded cancellous screw (DePuy Synthes, Oberdorf, Switzerland) was then used for interfragmentary compression (as lag screw) of the fractures in the FULL group while the 3.5-mm partially-threaded screw

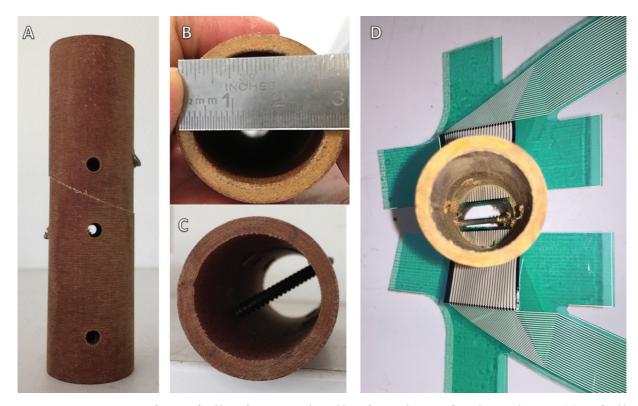
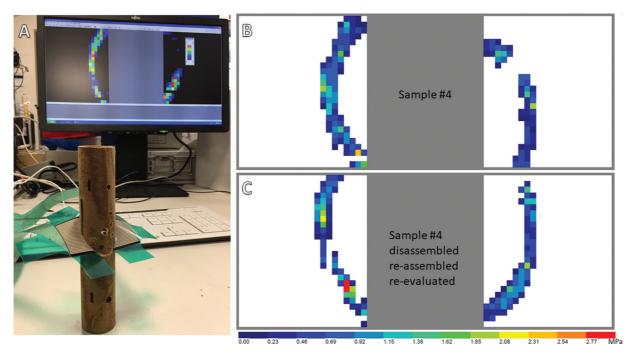


Fig. 1 Test construct consisting of two artificial bone fragments with an oblique fracture line were fixated using a lag screw (A). Artificial bone models were created from hard composite fiber tube with outer diameter 30 mm, and inner diameter 20 mm (B). A fully threaded (C) or a partially threaded screw (D) was placed perpendicular to the fracture line through the center of the tube. Flat pressure sensor foil was placed between the two fragments to measure the interfragmentary compression (D).



**Fig. 2** Pressure distribution (force per area) was measured with an interfragmentary sensor, Tekscan 4000, **(A)**. Repeatability is shown for one sample #4, **(B)** versus **(C)**, same sample, disassembled, reassembled and reevaluated. Pressure integral was used to evaluate the compression force. The sensor was removed for biomechanical testing.

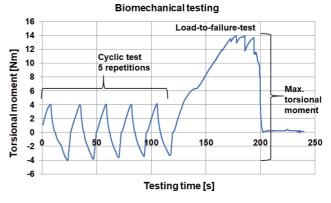
(DePuy Synthes, Oberdorf, Switzerland) was used for interfragmentary compression (as lag screw) in the PARTIAL group (**Fig. 1**). Core diameter of the screws was 2.4 mm. Screw material was stainless steel.

#### **Pressure Sensor Measurement**

Interfragmentary pressure was recorded using Tekscan 4000 pressure mapping sensors (Tekscan, Inc., Boston, MA, USA). The sensors were preconditioned and calibrated using a universal mechanical testing machine (Z010 - ZwickRoell, Ulm, Germany). Qualitative pressure distribution (**Fig. 1D**, **Fig. 2**) and total force as integral of pressure over area were evaluated.

#### **Biomechanical Testing**

Biomechanical testing in torsion (Z010 - ZwickRoell) of the samples was subsequently performed after loosening of the screw (disassembly), removal of the sensor, and reassembly (re-tightening of the screw). Initially, cyclic tests in internal/external rotation were run. The construct was rotated clockwise at a rate of 20°/min to 3 degree rotation (or 4 Nm torque); then, the construct was rotated counter-clockwise at a rate of 20°/min to -3 degree rotation (or -4 Nm torque) while sustaining an axial load of 10 N. Torsional stiffness [Nm/deg] for both rotational directions was evaluated based on the angle-moment curve as well as on the time-moment curve for 50% of the achieved maximum values, that is, angle and moment, respectively. After 5 repetitions, a load-to-failure test was conducted at a rate of 20°/min and up to 45° rotation or failure (clockwise). Maximum torsional moments [Nm] were evaluated, Fig. 3.



**Fig. 3** Biomechanical test loading protocol example showing 5 repetitions of loading up to 4 Nm torque in both directions followed by load to failure (45-degree rotation or drop-in moment).

#### Micro-CT Scan

A micro-CT measurement (VivaCT40, Scanco, Switzerland) with voxel size of edge length of 0.038 mm was performed on one sample from each group (**Fig. 4**) to evaluate contact area of the screw with the artificial bone using the 3D-visualization and processing software Amira 2016.51 (Zuse Institute Berlin, Thermo Fisher Scientific).

#### **Statistical Analyses**

We started with 12 samples, but 3 samples had to be excluded from the analysis of the biomechanical test results: interfragmentary compression force plots were analyzed based on the interquartile range (IQR)-method (> 1.5\* IQR deviation) to ensure consistent compression (**> Fig. 5A**), and 2 outliers in the PARTIAL group were excluded, while the first sample in the FULL group showed problems with data

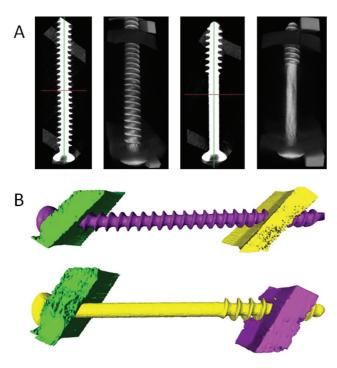


Fig. 4 Micro-CT scan results showing (A) from left to right: fully threaded screw cross section, fully threaded screw 3D reconstruction, partially threaded screw cross section, partially threaded screw 3D reconstruction, (B) top: fully threaded screw fixation in Amira software 3D reconstruction, (B) bottom: partially threaded screw fixation in Amira software 3D reconstruction.

acquisition during biomechanical testing, resulting in a final sample size of 9 constructs (FULL n = 5, PARTIAL n = 4). Both groups were compared based on the biomechanical parameters; angle-moment-stiffness-clockwise, angle-momentstiffness-anti-clockwise, time-moment-stiffness-clockwise, time-moment-stiffness-anti-clockwise, maximal torsional moment, and calibrated compression force (N=11, loss during data collection in the PARTIAL group). Recorded quantitative data for all biomechanical parameters were checked for normality using kernel-density-estimation (KDE) plots. 15 Upon analysis of the KDE plots for all abovementioned biomechanical parameters, it was determined that the data was non-parametric in nature. Moreover, one of the measurements for calibrated compression force for partially-threaded screws was lost during data collection reducing the sample size for calibrated compression force for the partially-threaded screw group to n = 3. The resulting data was then analyzed using descriptive statistics and nonparametric mean comparisons (Mann-Whitney-U-Test) to compare groups of screws. Furthermore, Spearman correlation analyses were performed to determine associations between biomechanical parameters mentioned above.

## Results

#### **Pressure Sensor Measurement**

No statistically significant differences in calibrated force measurements were observed between FULL and PARTIAL lag screw groups [median (IQR) for FULL: 112.6 (10.5) N,

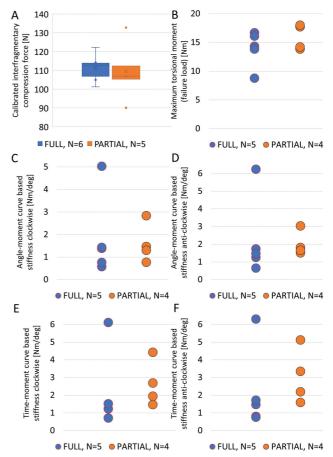


Fig. 5 Box plot of compression force results and scatter plots of biomechanical results. Evaluated sample sizes are given as N. Please note that one sample in the PARTIAL group was lost due to data acquisition error in the compression force measurement (A). Furthermore, one sample in the FULL group was lost due to data acquisition problems during biomechanical testing, and two samples were excluded in analysis (PARTIAL) of the biomechanical data (B-F) after evaluation of compression force results (A) to ensure consistent compression. Subplots show: (A) Calibrated interfragmentary compression force [N], (B) Maximum torsional moment (failure load) [Nm], (C) Angle-moment curve-based stiffness clockwise [Nm/deg], (D) Angle-moment curve-based stiffness anticlockwise [Nm/deg], (E) Time-moment curve-based stiffness clockwise [Nm/deq], and (F) Time-moment curve-based stiffness anti-clockwise [Nm/deg].

PARTIAL: 106.9 (7.1) N, p = 0.79, **Table 1**, **Fig. 5A**]. The repeated measurement of one sample (sample #4, disassembled, reassembled) resulted in similar pressure distribution (►**Fig. 2B,C**) and a total force difference of 0.35%.

# **Biomechanical Test**

Descriptive statistics were calculated as (IQRs), -Table 1. No statistically significant differences in maximum torsional moment were observed between the FULL and PARTIAL groups (FULL: 14.37 (5.08) Nm, PARTIAL: 15.98 (4.00) Nm, p = 0.79, **Table 1**, **Fig. 5B**). No statistically significant differences were observed between the FULL and PARTIAL groups in angle-moment-stiffness-clockwise (FULL: 1.39 (2.56) Nm/deg, PARTIAL: 1.39 (1.59) Nm/deg, p = 0.73, **Table 1**, **Fig. 5C**) and anti-clockwise (FULL:

Biomechanical test	Screw type (group)	Median	Interquartile range	Mann-Whitney-U test
				Exact sig. (2-tailed) p-value
Calibrated interfragmentary compression force [N]	Full	112.6	10.5	0.79 <sup>a</sup>
	Partial	106.9	7.1	
Maximum torsional moment (failure load) [Nm]	Full	14.4	5.1	0.56ª
	Partial	16.0	4.0	
Angle-moment curve-based stiffness clockwise [Nm/deg]	Full	1.4	2.6	0.73 <sup>a</sup>
	Partial	1.4	1.6	
Angle-moment curve-based stiffness anti-clockwise [Nm/deg]	Full	1.5	3.0	0.41 <sup>a</sup>
	Partial	1.7	1.2	
Time-moment curve-based stiffness clockwise [Nm/deg]	Full	1.2	3.1	0.29 <sup>a</sup>
	Partial	2.3	2.4	
Time-moment curve-based stiffness anti-clockwise [Nm/deg]	Full	1.5	3.2	0.29 <sup>a</sup>
	Partial	2.8	2.9	

**Table 1** Descriptive data for various biomechanical analyses of FULL and PARTIAL lag screw fixation in artificial bone samples

Mann-Whitney U-test was used to determine the presence of significant differences between groups.

1.47 (3.03) Nm/deg, PARTIAL: 1.74 (1.18) Nm/deg, p=0.41, **FTable 1**, **FFig. 5D**). No statistically significant differences were observed between the FULL and PARTIAL groups in time-moment-stiffness-clockwise (FULL: 1.23 (3.10) Nm/deg, PARTIAL: 2.32 (2.41) Nm/deg, p=0.29, **FTable 1**, **FFig. 5E**) and anti-clockwise (FULL: 1.47 (3.23) Nm/deg, PARTIAL: 2.78 (2.94) Nm/deg, p=0.29, **FTable 1**, **FFig. 5F**).

To determine associations between the various biomechanical parameters, Spearman correlations were calculated among all biomechanical parameters. Spearman correlations that were significant at the 0.05 and 0.01 levels were assessed. With regards to fully-threaded screws, strong significant positive correlations were determined between angle-moment-stiffness-clockwise and angle-moment-stiffness-anti-clockwise (r = 0.9, P < 0.05), angle-moment-stiffness-clockwise and time-moment-stiffness-clockwise (r = 0.9, P < 0.05), and angle-moment-stiffness-clockwise and time-moment-stiffness-anti-clockwise (r = 0.9,P < 0.05). The significant associations mentioned above were not observed for partially-threaded screws. However, a strong significant positive correlation was observed between time-moment-stiffness-clockwise and time-moment-stiffness-anti-clockwise (r = 1.0, P < 0.01) for both FULL and PARTIAL groups.

#### **Micro-CT Scan**

The contact area between artificial bone and screw at the far (trans)-cortex where thread is present for both screws was 69.9 mm<sup>2</sup> and 69.4 mm<sup>2</sup> for fully and partially threaded single screw samples. In contrast, the contact area at the near (cis)-cortex (near screw head, not only the threaded or

non-threaded screw shaft area), where only the fully-threaded screw exhibits a thread, was  $60.8\,\mathrm{mm^2}$  and  $44.9\,\mathrm{mm^2}$  for the fully and partially-threaded single screw samples, respectively. The near cortex contact area is, thus,  $\sim$  35% larger with a fully-threaded screw compared with a partially-threaded screw.

#### Discussion

We conducted a biomechanical pilot study to assess differences between fully-threaded and partially-threaded screws in the initial interfragmentary compression strength and construct stiffness. We hypothesized a detectable loss of initial compression strength (lower interfragmentary compression force, and lower failure load in torsion) and lower construct stiffness with the partiallythreaded screw, as there is no screw thread contact across the near (cis-)fracture fragment. In a study comparing transarticular screw fixation for Lisfranc injury, the difference of fully-threaded solid cortical (FSC) and partiallythreaded cannulated cancellous (PCC) screws provided equal amounts of fixation strength during partialweight-bearing and similar resistance to deformation under bending loads. 16 In a synthetic Schatzker type 1 tibia fracture model, addition of a fully-threaded screw was tested against addition of a second partially-threaded screw next to a first partially-threaded screw over a compressed fracture. No significant differences between the two groups in terms of failure were found, although it was also found that presence of one fully-threaded screw can minimize displacement at the fracture site at early cyclic loadings.17

<sup>&</sup>lt;sup>a</sup>Mann-Whitney U-test grouping variable: FULL versus PARTIAL.

The exact quantification of compression force and failure load in torsion (compression strength), as well as construct stiffness, is relevant, because the measured compression forces (pull-apart forces) around 100 N here and in a previous ovine model 18 or for other screw types of similar size in foam blocks 19 could be overcome by physiological tension loading in bending or torsion, necessitating, for instance, tensionband plating, additional lag screws, larger screws, or absolutive stability. 18 We did not find statistically significant differences in interfragmentary compression force, potentially due to low power or the technique we used, which, similar to clinical practice, does not use a torque-meter nor ensures perfect perpendicular screw insertion. The median differences between the groups are  $\sim$  5% of the achieved compression forces, while standard variations could be  $\sim 5$  to 10% of the compression forces or even higher considering all tested samples. A power analysis to estimate the needed sample sizes for significant differences revealed (assuming mean 106.9 versus 112.6, standard deviation 7.1,  $\alpha$  0.05, power 0.8) that a minimum of n = 24 samples per group would be required. The differences in stiffness, up to  $\sim 90\%$ median difference, and in failure load, up to  $\sim 10\%$  difference, are larger than for compression force, but they are also accompanied by much larger variance.

The total construct stiffness results from the out-of-plane bending of the screw and the interaction at the screw-bone interfaces. The interaction at the near (cis-)fragment with a fully-threaded screw might indeed be stronger and stiffer, especially as we strictly over-drilled both groups. A fullythreaded screw exhibits a smaller effective diameter due to the reduced diameter between the threads compared with a partially-threaded screw with a smooth screw shaft. As bending stiffness increases with diameter to the fourth power, even small differences in effective screw shaft diameter increase the screw's bending strength, and this leads to reduced resistance to bending for the fully-threaded screws in the shaft area. In cases when a screw bridges a gap without achieving interfragmentary compression (non-lag), there is also the competing influence of the working length (free bending length) of the screw, which would be higher for a partially-threaded screw, which mostly anchors at the screw head, while a fully-threaded screw can hold on to both inferior and superior fragment sites close to the gap. Miles et al.<sup>20</sup> compared mean displacement during cyclic testing of lag versus non-lag screw fixation in oblique finger fractures in a cadaver model and did not find significant differences. Resulting shear forces might be more detrimental to partially-threaded screws for long screw working length, but with very short screw working length, the smaller effective screw shaft diameter in fully-threaded screws may lead to higher screw stresses amplified due to the notch factor (stress riser). This becomes relevant also in locking screw fixations (nonlag), when screw working length is utilized for instance in far cortical locking to adapt interfragmentary stiffness, but this requires adapted screw dimensions.<sup>21,22</sup> In fully vertically unstable transforaminal sacral fractures, threaded transiliactranssacral (TI-TS) fixation was compared with partially threaded TI-TS fixation. The results indicate, for this cadaveric biomechanical analysis in an unstable fixation, that displacement and failure rate were higher for partiallythreaded specimens, and fully-threaded specimens demonstrated greater mean force to failure.<sup>23</sup> In a synthetic sawbone block loaded in shear, yield load and displacement of fully-threaded screws were 64% and 67% greater than those for partially-threaded screws, respectively.<sup>2</sup>

In our study, as well as in previous ones, the ranges of the observed parameters, such as compression force, were large (standard deviation up to 9% of the mean in our study, and up to 12%), <sup>19</sup> and we excluded the extreme cases (of interfragmentary compression force) in the statistical analysis, but the fact that there are outliers should be stressed (>Fig. 5), and such broad variation with outliers has been described before.<sup>24</sup> Medians in compression force and torsional stiffness were generally lower and associated with larger variance in the FULL group (not significant, low power). Those factors of high variance are most likely the product of the surgical technique and possibly of the constructs themselves: fully-threaded screws might be more difficult to place reliably as the contact between near cortical pilot hole and screw is specimen-specific and not very easy to standardize, so partially-threaded screws may simplify the operative procedure and minimize nonoptimal screw placement.<sup>16</sup> However, theoretically, we expect that fully-threaded screws might lead to slightly higher failure loads in high-density bone <sup>19</sup> through more contact area, which we could not prove statistically in this pilot study. In the micro-CT assessment, we could show a 35% higher contact area at the near (cis-) fragment close to the screw head for a fully-threaded lag screw compared with a partially-threaded lag screw. We could show similar interfragmentary compression forces for the FULL and PARTIAL groups.<sup>4</sup> Possibly, there is still some toggling and contact, especially in long narrow canals of comparably stiff substrate, and, perhaps as a result, the fullythreaded screws should be used with wider over-drilled holes in the near (cis-)fragment, much larger than the outer diameter, to achieve even higher compression forces. The difference in compression force between fully and partially threaded screw fixation might be more pronounced in weaker, less dense bone if there is a diminished contact area around the screw head as demonstrated in the micro-CT assessment.

In this biomechanical study, a high-density bone model was used, similar to cortical bone. The impact on a softer osteoporotic or metaphyseal bone model is not known; hence in such models, such a fixation may lead to a loss of reduction. Fully-threaded screws could, therefore, be useful in diaphyseal fracture treatment in younger patients with better bone quality. The limitations of the method to measure interfragmentary pressure and compression force have been extensively discussed in a previous publication. 18 Disassembly and reassembly between pressure measurement and the biomechanical testing have been shown to be reproducible in terms of compression force and general pressure distribution (>Fig. 2), but the high variance within groups might be a result of this reassembly. Furthermore, to avoid such outliers, the process of creation of compression would probably have to be standardized more rigorously, that is, with guide systems for the drilling process, the screw application, and guide blocks for the fracture fragments. However, this would not represent the clinical application anymore. Limited sample size may have resulted in the absence of statistical differences between groups; however, the median differences between the groups were small, and variability was comparably high within groups as well. We tested a stable configuration with verified interfragmentary compression. It has been shown before that fully-threaded screws might be superior compared with partially-threaded screws in an unstable configuration, that is, without interfragmentary compression such as with a remaining gap (positional screw). The biomechanical test setup in torsion might not be representative of the clinical reality as, clinically, lag screws must be used either in combination with a neutralization plate or at least another interfragmentary compression screw when torsion or bending are encountered.

#### **Conclusions**

There is no apparent difference in the initial compression strength (failure load in torsion) achieved using either fullyor partially-threaded screws in this stable, high-density artificial bone biomechanical model. Further studies are required to evaluate the clinical significance of this finding.

#### **Authors Contributions**

All authors contributed to the study conception and design. Material acquisition, preparation and data collection were performed by T. O., M. L., G. R., D. W., and M. H. Data analysis was performed by T. O., E. A., and M. H. The first draft of the manuscript was written by T. O., E. A., M. H. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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#### **Conflict of Interests**

The authors have no conflict of interests to declare.

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