Differences in the Mechanical Properties of Calcaneal Artificial Specimens, Fresh Frozen Specimens, and Embalmed Specimens in Experimental Testing

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ABSTRACT

Background. Artificial calcanei, fresh-frozen cadaver specimens, and embalmed cadaver specimens were compared in experimental testing under biocompatible loading to clarify the biocompatibility of artificial calcaneal specimens for implant testing. Methods. Two different artificial calcaneal bone models (Sawbone™, Pacific Research Laboratories, Vashon, WA, and Synbone™, Synbone Inc., Davos, Switzerland), embalmed cadaver calcaneal specimens (bone density, 313.1 ± 40.9 g/cm²; age, 43.8 ± 7.9 years), and fresh-frozen cadaver calcanei (bone density, 238.5 ± 30.0 g/cm²; age, 44.4 ± 8.2 years) were used for testing. Seven specimens of each model or cadaver type were tested. A mechanical testing machine (Zwick Inc., Ulm, Germany) was used for loading and measurements. Cyclic loading (preload 20 N, load was increased every 100 cycles by 100 N from 1,000 to 2,500 N, 0.5 mm/s) and load to failure (0.5 mm/s) were performed. The loads were applied through an artificial talus in a physiological loading direction. The displacement of the posterior facet in the primary loading direction was measured. Results. The four different specimen groups showed different stability and different displacement in the primary loading direction during cyclic loading. The variation of the maximal displacement in the primary loading direction for the entire cyclic loading was higher in artificial specimens than in the cadaver specimens. Conclusions. Artificial calcanei (Sawbone™, Synbone™) showed different biomechanical characteristics than cadaver bones (embalmed and fresh-frozen) in this experimental setup with biocompatible cyclic loading. These results do not support the use of artificial calcanei for biomechanical implant testing. Fresh-frozen and embalmed specimens seem to be equally adequate for mechanical testing. The low variation of mechanical strength in the unpaired cadaver specimens suggests that the use of paired specimens is not necessary.

Key Words: Artificial Specimen; Cadaver Specimen, Calcaneal Specimen; Mechanical Properties

INTRODUCTION

Artificial bones have widely been used for implant testing.1,11,17–19,21,26 These are, in general, of questionable biocompatibility because the internal architecture, and resulting directional mechanical properties of the real bone are absent.9 However, these specimens have identical size and accurate morphology and are considered to have a very low variability of strength.18,31 These features may make them preferable to cadaver bone, which have high variability in their size and quality and are considered to have a high variability of strength.15 Only paired specimens from the same cadaver are considered to have comparable biomechanical characteristics to allow simultaneous comparison of two different implants.15 If more than two different implants are compared at a time, the use of cadaver specimens is possible only if a problematic sequential testing of different implants with the same specimen is performed.23 In the special situation of implants designed for the treatment of calcaneal fractures, the biocompatibility of the cadaver bones also is debatable, because most of these specimens are taken from individuals with a mean age of 80 years.2,3,15,23 In contrast, the mean age of patients with calcaneal fractures is 35 to 40 years.5,22,24,25,32 This age issue seems also to be important for implant testing because of the age-related bone density that is crucial to the result of implant testing.2 Still, calcaneal cadaver specimens of advanced age also have been used for implant testing.1–3,12,13,15,16,27 A mechanical comparison of the different calcaneal specimen types that takes into consideration a comparable age of cadaver specimens with the typical injury situation has not been done. In this study,
comparison of artificial calcanei, fresh-frozen cadaver specimens, and embalmed cadaver specimens in experimental testing under biocompatible loading was intended to clarify the biocompatibility of artificial calcaneal specimens for implant testing.

MATERIALS AND METHODS

The study was approved by the Ethical Commission of the Hannover Medical School, Hannover, Germany.

Specimen
Two different artificial calcaneal bone models (Sawbone™, Pacific Research Laboratories, Vashon, WA, USA and Synbone™, Synbone Inc., Davos, Switzerland), embalmed cadaver calcaneal specimens, and fresh-frozen cadaver calcaneal specimens were used for testing (Figure 1). No paired cadaver specimens were used. All specimens were tested at room temperature. The fresh-frozen specimens were thawed before the measurements and testing sequence. Seven specimens of each specimen type were tested. The number of tested specimens was determined by a statistician, by evaluation of the study design, and by a power analysis. The power of all statistical tests of the cyclic loading testing sequence for the determined sample size was more than .8. We attempted to use cadaver specimens that were “age-matched” with the observed age of individuals sustaining calcaneal fractures.5,22,24,25,32 Table 1 indicates the geometric sizes, age, and bone density of the different specimen groups. The bone density was measured by peripheral quantitative computed tomography (pQCT) as previously described.6,10,14 The Boehler and Gissane angles were measured on standardized lateral C-arm images. The cadaver specimens showed a high variation in geometric size and bone density. The weight of the specimens was not measured and compared, because the influence of the varying fluid content of the cadaver specimens was considered to be a biasing factor that could not be influenced.

Mechanical Testing Machine
A hydraulic testing and measuring machine (model Zwick 1445; Zwick Inc., Ulm, Germany) was used for loading and force and motion analysis23 (Figure 2). The specimens were embedded with their posterior process fixed using standard veterinary bone cement (Demotec 95, Demotec Inc., Nidderau, Germany) (Figure 3). This cement has the same ingredients and properties as that used for humans (Palacos; Biomet Merck Inc., Berlin, Germany). However, it is of

![Fig. 1: Calcaneal specimens for biomechanical testing. A, Sawbone™ specimen (Pacific Research Laboratories, Vashon, WA, USA). B, Synbone™ specimen (Synbone Inc. Davos Switzerland). C, Embalmed cadaver specimen. D, Fresh-frozen cadaver specimen.](image-url)
low cost. A calcaneal inclination angle of 20 degrees and a neutral position regarding a supposed hindfoot angle were achieved, simulating the normal angles and position. The angles (calcaneal inclination, supposed hindfoot angle) were controlled with a digital goniometer (Winkelmesser-DIGIT™, Gottlieb Nestle Inc., Dornstetten, Germany; accuracy, ±1 degree). The load was applied and transmitted through artificial talar models that were incorporated into the testing machine. These models were made of a special metal-like material (Magic Bond Epoxydicht™, ITW Devcon Industrial Products Inc., Kiel, Germany). The talar models were shaped by impressions from the talus of the cadaver specimens or from standard talar models from the artificial bone specimen manufacturers. One individual talus was used for each cadaver specimen, one talus was used for all Sawbone™ specimens, and one talus was used for all Synbone™ specimens. A physiological alignment between the talus and the calcaneus was ensured. The testing machine was controlled by a standard IBM compatible personal computer with control software installed (model test-expertSoftware; Zwick Inc., Ulm, Germany). The mechanical testing machine measured the displacement of the testing machine head. This was considered to be equivalent to the displacement of the specimen surface of the posterior facet. The measured data were directly transferred to the same computer. All data were exported and stored in ASCII files for further statistical analysis.

**Motion Analysis System**

The spatial orientation of the specimen, and the plate were recorded with an ultrasound measurement system (model CMS HS; Zebris Inc., Tuebingen, Germany). The sound transducers were included in the measurement system (cylindrical shape; height 10 mm; diameter, 5 mm; weight, 1 g). The absolute spatial accuracy of the system was rated as 0.1 mm, resolution as 50 um and the angular accuracy for the triaxial sensors as less than 1 degree in all 6 degrees of freedom. This was reported by the manufacturer and independently in the literature.24,30 Two different measurements were performed.

1. The anterior process, the inferior margin and superior margin of the lateral wall and the posterior process were equipped with single transducers (uniaxial) and stickers (model Beidseitiges Klebeband; Zebris Inc., Tuebingen, Germany) (Figure 2).

   2. The posterior facet and the posterior process were equipped with triaxial transducers. The transducers were situated at the edges of an equilateral star-shaped adapter (model Plexiglas stern; Workshop, Hannover Medical School, Hannover, Germany, made of Plexiglas™, Rohm and Haas, Philadelphia, PA, USA) with a side length of 50 mm. The adaptors were fixed to the bone with Kirschner wires (model 2.0 mm Titan-K-Draht, Synthes Osteosynthese Inc., Bochum, Germany) (Figure 2). The triaxial marker that was inserted in the specimen beneath the posterior facet was used to measure the displacement of the posterior facet.

   The motion analysis system failed after 23 testing sequences and was not repairable. However, after this failure there were no alterations to the constructs for the remaining specimens.

**Testing Sequences**

Following the specimen construction, the mechanical testing machine and the motion analysis device were started,
and the testing sequences were performed.

1. Cyclic loading (preload 20 N, load increasing every 100 cycles by 100 N from 1,000 to 2,500 N, 0.5 mm/s) was applied (1,600 cycles in total).
2. Load to failure at 0.5 mm/s followed.

The rationale for the progressive cyclic loading was based on experience from an earlier study. During that study, Sawbones™ with a calcaneal fracture model and different calcaneal plates were loaded for 1,000 cycles with 800 N. Sawbones without a fracture model or plate were tested as a control group with the same loading. The displacement of the specimens of the control group was very low in that testing sequence. Therefore, we found that a higher load should be applied. Since we did not know the correct load, we chose a progressive load. The motion analysis system recorded the readings of the first, tenth, every 100th, and 1000th cycles and the load to failure. Failure (endpoint) was defined as a further deformation of the specimen and a decrease of the load at the same time, i.e., a rapid decrease of the load/deformation graph (load, y-axis; deformation, x-axis) resulting in the specimen being unable to take anymore load. Failure (endpoint) also was defined as a displacement of more than 3 cm in the primary loading direction. For safety reasons, the maximal force was limited to 5,000 N. The measurements during cyclic loading and the load to failure sequences were considered to be a parameter for the sum of elastic and plastic deformation. After the entire testing sequence, the specimens, including the transducers, were removed from the testing machine. The constructs were examined independently by two senior orthopaedic trauma surgeons (S.Z. and T.G.) and remarks concerning failures were recorded (Table 2, Figure 3). The remaining joint depression and the decrease of Boehler angles were recorded. The Boehler angles were measured with an electronic goniometer (Winkelmesser-DIGIT™, Gottlieb Nestle Inc., Dornstetten, Germany; accuracy, ±1 degree). The remaining joint depression was measured with an electronic caliper (Absolute Digimatic™, Mitutoyo Inc. Germany, Neuss, Germany; accuracy, ±0.001mm). These measurements were considered to be a parameter for plastic deformation. The remaining joint depression was measured at the specimen in a standard fashion with an electronic caliper. In three cases the remarks concerning failures differed. Both evaluators repeated their evaluation regarding the remarks concerning failures. The second evaluations did not differ.

Statistical Analysis and Hypothesis Testing
One-way ANOVA was used for comparison of measurements. When significant differences occurred during the ANOVA-test, a Post-Hoc Scheffé was used to locate the differences between the different specimen-plate constructs. Pearson test was used for correlation between measurements of the mechanical testing machine and the motion analysis device and for correlation between measurements of load to failure and of cyclic loading. The null hypothesis at the p < .05 level was that there is no difference between the different specimen groups.

RESULTS
Specimen no. 22 (fresh-frozen cadaver) failed during cyclic loading cycle 1,193 (2,100 N). Therefore, this specimen did not complete the cyclic loading cycles 1,194 to 1,600, and load to failure (Tables 2 and 3). The analysis of cyclic loading (all cycles, cycles 1,596–1,600, difference of cycles 1,596–1,600 minus 1–5) and of load to failure included only 27 specimens six fresh-frozen specimens. Failures of the test fixation of the specimens were not observed.

Mechanical Testing Machine
The following features that were shown to be statistically significant are presented in order of significance.

1. Cyclic loading: All cadaver specimens showed a statistically significantly higher displacement in the primary loading direction than the artificial specimens (Tables 2 and 3, Figures 4, 5, and 6). Therefore, the null-hypothesis was rejected. The variation of the “maximal displacement” in the primary loading direction for the entire cyclic loading was higher in artificial specimens than in cadaver specimens (Figure 5). This means that the “maximal displacement” during the entire cyclic loading increased more for artificial specimens.
Table 2: Testing protocol and results of the mechanical testing machine. The mechanical testing machine measured the displacement of the testing machine head. This was not considered to be equivalent to the displacement of the specimen surface of the posterior facet.

<table>
<thead>
<tr>
<th>No.</th>
<th>Specimen</th>
<th>Motion analysis</th>
<th>Load to failure (N)</th>
<th>Failure pattern/ fracture type</th>
<th>Remaining decrease Boehler’s angle (degrees)</th>
<th>Remaining joint depression (mm)</th>
<th>Irregularities during testing</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Sawbone™</td>
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<td>4,206</td>
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<td>Maximum load of mechanical testing machine exceeded</td>
</tr>
</tbody>
</table>

(Continued)
Table 2: (Continued)

<table>
<thead>
<tr>
<th>No.</th>
<th>Specimen</th>
<th>Motion analysis</th>
<th>Load to failure (N)</th>
<th>Failure pattern/ fracture type</th>
<th>Remaining decrease Boehler’s angle (degrees)</th>
<th>Remaining joint depression (mm)</th>
<th>Irregularities during testing</th>
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</table>
than for cadaver specimens. The mechanical strength of the cadaver specimens remained more stable; less displacement occurred than in the artificial specimens. However, since one specimen from the “fresh-frozen” group failed during the cyclic loading, this entire group may have an overestimated stability for the later cycles after failure and for load to failure. When analyzing the last 100 cycles with all specimens (cycles 1,001 — 1,100; 2,000 N), before failure of specimen 22, the cadaver specimens still showed a statistically significantly lower displacement \( p = .01 \) in the primary loading direction than the artificial specimens (Figure 6). The variation of the displacement in the primary loading direction among these 100 cycles did not differ significantly between the different specimen types; the variation of the mechanical strength within the artificial specimen groups was not lower than in the cadaver specimen groups (oneway ANOVA of standard deviations, \( p = 0.23 \)).

2. Load to failure: The measurements for the displacement during load to failure were higher in the Synbone\textsuperscript{™} than in the Sawbone\textsuperscript{™}, and higher in both than in cadaver specimens (Table 3). The fresh-frozen specimens did not differ from the embalmed specimens. The remaining decrease of the Boehler angle was higher in the Synbone\textsuperscript{™} than in the Sawbone\textsuperscript{™}.

**Fig. 4:** Error bars with 95% confidence interval of maximal displacement in the primary loading direction of the different specimen groups during cyclic loadings (all cycles).

**Fig. 5:** Boxplots of maximal displacement in the primary loading direction of the different specimen groups during cyclic loadings (all cycles).

**Fig. 6:** Boxplots of maximal displacement in the primary loading direction of the different specimen groups during cyclic loadings (cycles 1,001 — 1,100; 2,000 N).
and the embalmed cadaver specimens. The differences of the maximal load during load to failure and remaining joint depression failed to reach statistical significance. There was no significant correlation between the results of load to failure sequence (maximal load, motion amplitude during load to failure, remaining decrease of the Boehler angle, and remaining depression of the posterior facet) with those obtained from the cyclic loading sequence (Pearson, $p > 0.05$).

**Motion Analysis System**

The readings of the motion analysis system before failure were only available for 23 specimens. In these, the measurements from the marker that was fixed onto the superior margin of the lateral wall beneath the posterior facet (see above) correlated with that of the mechanical testing machine (Pearson, $r > 0.8$, $p < 0.001$).

**Examination of Specimens**

The examination of the specimens at the end of testing revealed that specimens tested shared similar failure patterns (Table 2). A fracture of the anterior process and joint depression fracture type according to the Essex-Lopresti classification was the most frequent failure pattern (Table 2, Figure 3).8

**DISCUSSION**

The aim of this study was to test the relative strength of different specimen types currently in use for implant testing. The load was designed to mimic the forces transmitted through the calcaneus during standing. However, this design had several shortcomings. We did not measure the torque forces that may be produced in vivo by muscular contraction and ligamentotaxis, which are of importance during standing.
walking and climbing stairs. The tested calcaneal specimens were positioned in a similar fashion to those in living individuals in an upright position.\textsuperscript{32} These constructs lack the force applied by tendons and ligaments normally present in physiological status. The effect of these soft tissues has been suggested to influence calcaneal fracture patterns similar to the effect described for the Achilles tendon in tongue-type fractures.\textsuperscript{5} However, there is no evidence to support these effects.

The results of the motion analysis were difficult to interpret because of relevant artifacts within the measurements. These artifacts were mainly caused by the close and sound-reflecting surfaces of the mechanical testing machine. This problem could not be eliminated despite extensive efforts and different modifications such as covering the mechanical testing machine with towels. Furthermore, the entire system failed during the study. However, measurements obtained from the primary loading axis also were registered by the mechanical testing machine. The readings of both systems were analyzed and found to be comparable. Data extracted from the mechanical testing machine were considered to be sufficient for the analysis of the final results. We report these findings because we believe that our experience with the motion analysis system will be helpful for other expert groups that perform or want to perform experimental testing comparable to ours. The most important finding is that the motion measurements of the mechanical testing machine are equivalent to those of the motion analysis system. For the future, in our or other institutions, the use of an additional motion analysis system for that kind of experimental testing does not appear to be necessary.

Implants and a fracture model were not included in our testing. The tested mechanical properties of the specimens did not include features like screw or wire fixation stability. We did not include implants or a fracture model because we doubt that an absolutely accurate standardization of a fracture model and implant application is possible. In an earlier study, we observed a variation of the "standardized" cuts of the fracture model and of the plate and screw fixation despite the use of artificial specimens with low inter-individual differences of specimen size and shape.\textsuperscript{23} We suspect that this variation of fracture model cuts and fixation would be even higher in cadaver specimens with high interindividual differences of size and shape. Any variation in an applied fracture model or fixation would, of course, affect the mechanical stability of the entire construct. Because we wanted to test the mechanical properties of different specimens, we decided not to include a fracture model.

We are not able to define possible different loading characteristics that might be caused by the cartilage on the cadaver specimens or the missing cartilage on the artificial specimens. Wong et al.\textsuperscript{29} reported that subtle differences of cellular processes of the cartilage can affect the micro- and macro-morphology of articular cartilage. This hypothesis was supported by in vivo and ex vivo experiments where load-induced changes in matrix synthesis and catabolism, gene expression, and signal transduction pathways were observed. Bommer et al.\textsuperscript{4} stated that different areas of articular cartilage are subjected to different types of loading and that differences in loading can adequately be met only when the tissue is biomechanically adapted to withstand these different loading conditions without injury. Oloyede et al.\textsuperscript{20} concluded that physiological rates of loading reduces the depletion of lipids from the articular cartilage, which reduces its compliance by at least 25%. They inferred from their study that this degenerative stiffening is an important contributing factor in impairing the tissue’s load processing function in osteoarthritic joints. However, none of these studies analyzed the differences of mechanical loading in surface with or without cartilage. Therefore, we do not know if the cartilage on the cadaver specimens or the missing cartilage on the artificial specimens led to an alteration of the measurements. We also did not measure shear forces that might give some information about the influence of the surface.

The load-to-failure testing sequence was not possible as planned in 17 of 28 specimens. One specimen (No. 22) failed during the cyclic loading testing sequence and 16 specimens (No. 8—17, 21, 22—27) did not fail with the possible maximal load to failure force of 5,000 N. A load of 5,000 N was previously considered to be high enough for the load-to-failure testing sequence, and we found no information in the literature that this load would be insufficient. As far as we know, a load of 5,000 N was not reached in any testing sequence before. Based on the missing failure in 16 of 28 specimens, the results of the load-to-failure testing sequence are not of sufficient value. Nevertheless, when analyzing the results of the load-to-failure testing sequence, a significant correlation of these results with those from the cyclic loading testing sequence was not found. This finding confirms the findings from earlier studies with sufficient load-to-failure testing sequences.\textsuperscript{7,23} In those studies, the sufficient load-to-failure testing sequence was found to be of questionable value based on the missing correlation with the cyclic loading.\textsuperscript{7,23} In agreement with the literature, we believe that even a sufficient load-to-failure testing sequence would have been of much less conclusiveness than a sufficient cyclic loading testing sequence as achieved.\textsuperscript{7,23,28}

Despite these shortcomings, our setting appeared to be more appropriate than those previously described.\textsuperscript{7,15} Lin et al. only measured single load-to-failure as opposed to cyclic loading.\textsuperscript{15} Furthermore, only one type of cadaver specimen was used in their study, which has potential disadvantages as described earlier.\textsuperscript{15} Carr et al.,\textsuperscript{7} found comparable shortcomings even though fresh-frozen cadaver specimens were used. Despite the use of cyclic loading, the data recorded from their study were of questionable value since the loading was only 100 N.\textsuperscript{7} Cyclic loading appeared to be more appropriate than single load-to-failure, since the differences in the biomechanical behavior of the various specimen types were only detected by analyzing
the measurement of the cyclic loading.\textsuperscript{7,23,28} During the cyclic loading testing sequence the mechanical strength of the cadaver specimens remained more stable than that of the artificial specimens. However, since one specimen from the “fresh-frozen” group failed during the cyclic loading, this entire group may have an overestimated stability for the later cycles after failure and for load to failure. When analyzing the last 100 cycles with all specimens, before failure of the one specimen, the cadaver specimens still showed a statistically significantly higher displacement in the primary loading direction than the artificial specimens. The results of load-to-failure sequence (maximal load, displacement during load to failure, remaining joint depression, and remaining decrease of the Boehler angle) did not correlate with those from the cyclic loading sequence. The measurements of the remaining joint depression and remaining decrease of Boehler angle were considered as parameters for plastic deformation and the other measurements as parameters for the sum of plastic and elastic deformation. This explains the higher values of the maximal displacement during the cyclic loading and the load-to-failure sequences (sum of elastic and plastic deformation) than of the remaining joint depression and remaining decrease of the Boehler angle (plastic deformation).

The cadaver specimens were age-related to the injury situation for calcaneal fractures with a low variation of age.\textsuperscript{5,22,24,25,32} The cadaver specimens were not paired. We observed a high variation of geometric size, weight, and especially bone density among these specimens. The variation of the geometric size and weight was, of course, much higher than in artificial specimens. Surprisingly, the variation of the measured stability in the cadaver specimens was low and much lower than in the tested artificial specimens. Therefore, our earlier conclusion that the low inter-individual variation of size, shape, and weight in artificial specimens is advantageous because of supposed low interindividual differences of mechanical strength needs to be corrected.\textsuperscript{23} In contrast, the low variation of mechanical strength in unpaired cadaver specimens suggests that the use of paired specimens is not necessary. We could not demonstrate a difference in mechanical strength between fresh-frozen and embalmed specimens. Both specimen types seem to be equally adequate for mechanical testing.

To the best of our knowledge, this is the first biomechanical study to assess the mechanical properties of different calcaneal specimen types. Furthermore, this is the first attempt to analyze the stability of cadaver specimens that are age-matched to the observed age of victims sustaining calcaneal fractures in the real situation.\textsuperscript{5,22,24,25,32} Our results showed clearly that artificial calcaneal models (Sawbone\textsuperscript{TM} Synbone\textsuperscript{®}) showed different biomechanical characteristics than the age-matched cadaver bones (embalmed and fresh-frozen) in this experimental setup with biocompatible cyclic loading. The artificial calcaneal specimens showed different mechanical strengths despite their similar size and shape. The embalmed and fresh-frozen specimens did not differ in mechanical strength.

These results do not support the use of artificial calcaneal specimens for biomechanical implant testing.

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