

ORIGINAL RESEARCH

The Effects of Intramedullary Reaming on Residual Long Bone Biomechanical Properties in a Composite Femur Model

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ABSTRACT

Introduction: Intramedullary (IM) reaming of long bones without nail fixation has expanded to include bone graft harvesting and debridement for infection or tumor. This study evaluated the effects of IM reaming on the residual biomechanical properties of a composite femur model.

Methods: Eighteen composite femurs were sequentially reamed to 13, 14, 15, and 19 mm IM canal diameters using a trochanteric entry site. The reamed specimens were tested nondestructively in whole bone axial compression and torsion following each 1-mm reaming increment, and subsequently in four-point mid-diaphyseal bending. Finally, the femurs were subjected to failure testing in mid-diaphyseal four-point bending and proximal femur axial compression.

Results: A significant ($p \leq 0.0028$) linear decrease in axial compression stiffness of femurs was observed as IM reaming progressed to larger diameters. A reduction ($p \leq 0.0001$) in rotational stiffness of femurs was also noted. Mid-diaphyseal four-point-bending stiffness significantly decreased ($p \leq 0.0034$) for the larger reaming diameters (>16 mm), and exhibited a significant ($p < 0.0001$) difference between the medial-lateral and anterior-posterior planes. The largest IM reaming diameter (19 mm) reduced the load to failure ($p \leq 0.0052$) in both the diaphyseal and proximal femur testing modes; fractures consistently occurred through the trochanteric reaming entry site in the latter.

Discussion: Small-diameter (13-16 mm) IM reaming of a composite femur did not significantly reduce long bone stiffness and strength, but large-diameter (17-19 mm) IM reaming did. Large-diameter IM reaming via a trochanteric entry site made the proximal femur susceptible to fracture.

Keywords: Femur intramedullary reaming; Bone biomechanics; Intramedullary nail fixation.

INTRODUCTION

Intramedullary (IM) reaming of long bones is a common procedure typically preceding

IM nail fixation of a fracture. The IM reaming enlarges the IM canal to accommodate the largest possible nail, increases the contact area between the nail and cortical bone, and stimulates endosteal bone coupled with autografting to promote fracture healing [1]. Biomechanically, reamed IM nails provide better fracture fixation

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compared with unreamed nails [2]. However, depending on the residual outer and inner cortical bone diameters and the amount of bone removed, IM reaming diminishes the cortical thickness, thereby weakening the bone. Furthermore, the femoral reaming entry site to facilitate nail placement can also have deleterious effects upon the residual femur strength. The axial compression loads applied to the femoral head result in eccentric loading of the proximal femur, thereby creating a bending moment throughout the basic-cervical and trochanteric regions. In cadaveric femurs loaded in axial eccentric compression, the deviation of the entry site around the piriformis fossa exhibited a decrease in stiffness and load to failure of the proximal femur greater than the size of the entry portal [3].

Finite element analysis and composite femur testing demonstrated an exponential relationship between mechanical stiffness and cortical thickness for axial, torsion, and bending testing modes [4]. It is presumed that the removal of endosteal bone that occurs during IM reaming adversely alters long bone biomechanics and that these effects are typically compensated by the presence of an IM nail. Recently, however, the indications for IM long bone reaming as a stand-alone procedure without supplementary IM nail fixation have expanded to include autogenous bone graft harvesting or IM canal debridement for infection or tumor [5-9]. The extent to which a long bone can be reamed and retain its stiffness and strength sufficient to bear weight without supplemental IM nail placement is unknown. The objective of this study was to investigate the biomechanical effects of incremental IM reaming on residual bone stiffness and strength in a composite femur model. We hypothesized that femur IM reaming

without nail placement would compromise the femur's ability to withstand physiological loads.

MATERIALS & METHODS

Specimens and Reaming

Twenty medium-sized 4th-generation composite femur models (Model #3403; Pacific Research Laboratories; Vashon, WA, USA) were used. This composite femur model was redesigned to closely approximate the biomechanical properties of the human femur in fatigue resistance, as well as to have increased tensile and compressive strength [10]. The standard dimensions of the medium-sized composites are 455 mm in length, 27 mm in outer cortical diameter, and 13 mm in inner cortical diameter; the density of the solid foam core is 0.27 g/ml.

The composite femurs were reamed in 1-mm increments to final IM canal diameters ranging from 13 mm to 19 mm. Pilot testing was performed on an intact and a 19-mm-reamed femur to determine the elastic region loads for nondestructive stiffness testing, leaving 18 femurs for study testing and analysis. Antegrade IM reaming was performed via a trochanteric entry site, typical for most current femoral IM nailing applications as well as for IM autogenous bone graft harvesting. A 10-mm drill was used as an entry reamer and allowed the IM placement of a ball-tipped guide wire. Reaming was performed in a single-pass fashion starting at 13 mm. Each 1-mm reaming increment was accomplished with two passes of 0.5 mm increasing ream sizes to ensure a smooth cut through the synthetic canal. The composite femurs have an opening in the intracondylar notch

in line with the femoral canal, and reaming proceeded until the reamer had completely passed through this hole. All IM reaming was performed by the same orthopaedic surgeon.

Biomechanical Testing

Cyclic Nondestructive Testing

Specimens were tested nondestructively at 1-mm reaming increments in whole bone axial compression and whole bone torsion. Whole bone tests were performed to evaluate the biomechanical effects of sequential reaming (from 13 to 19 mm) on the femur subjected to simulated physiological loads. For the whole bone axial compression, the

specimens (n=18) were potted within custom fabricated polymethylmethacrylate (PMMA) clamps designed to fit the distal end of the specimen, with the shaft adducted 9 deg from the vertical axis to reproduce the physiologic biomechanical axis. The specimens were clamped into an 858 Mini-Bionix materials testing system (MTS; Eden Prairie, MN, USA), and the femoral head was loaded through a rail bearing, thus reducing the effects of coronal plane shear forces on the femoral head (Figure 1A). The femurs were loaded to approximate a body weight of 687 N (70 kg) under force control at a rate of 687 N/s (1 Hz) for 10 cycles.

For whole bone torsion testing, the specimens were oriented to replicate an-

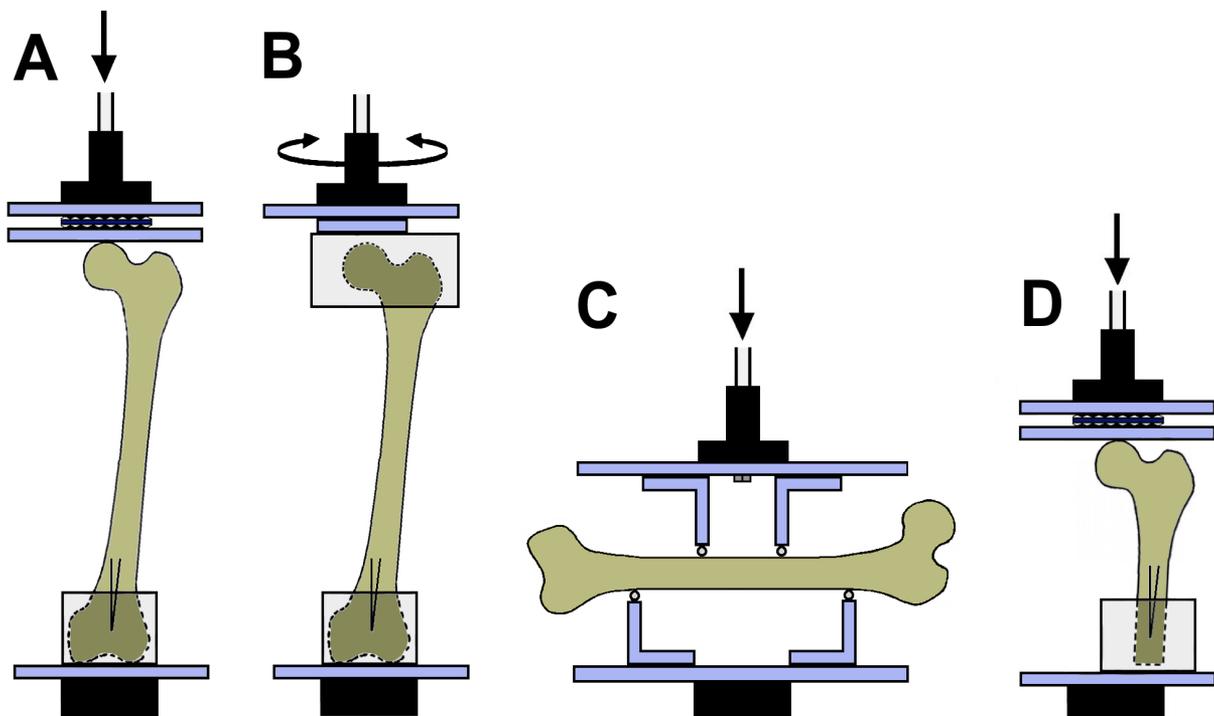


Figure 1. For whole bone axial compressive cyclic testing (A), the distal femur was rigidly fixed to the load cell at 9 deg and the femoral head loaded axially through a rail bearing. For whole bone torsional cyclic testing (B), the femur was stabilized proximally and distally while the actuator rotated internally and externally. For four-point bending cyclic and failure testing (C), the femur was placed between the supports in both medial-lateral and anterior-posterior planes for cycling, and in the medial-lateral plane for failure testing. For axial compressive failure testing of the proximal femur (D), the shaft was rigidly fixed to the load cell at 9 deg, and the femoral head loaded axially to failure through a rail bearing.

atomic 9 deg varus alignment and fixed distally in the same manner as for axial compression. After the specimens were clamped into the MTS machine, the proximal femur was fixed in an anterior-posterior manner about the femoral head and lesser trochanter with an adjustable clamp (Figure 1B). The femurs were then loaded in torsion to 10 Nm under force control in both internal and external rotation, at a rate of 1 Nm/s for 10 cycles.

After all 18 femurs had been reamed to their respective endpoints; the specimens were tested in four-point bending according to methods described elsewhere [11]. The femurs were aligned with the mid-diaphyseal region at the center of the loading jig mounted to the MTS machine. The lower supports were spaced 186 mm apart, and the upper load beams were 62 mm apart. The upper loading support was mounted on a swivel to allow the loading beams to maintain contact with the specimen throughout the loading cycle. The femurs were tested in medial bending (with the lateral surface in tension), and posterior bending (with the anterior surface in tension). Elastic bands were used on each end of the bottom supports and around the femur to prevent rotation on the clamp. Each femur was loaded to 500 N in a force control manner at a rate of 500 N/s for 10 cycles in the medial bending (Figure 1C), then repositioned and loaded in the posterior bending. No preloads were used for any of the cycling tests.

Load to Failure Testing

After the completion of all nondestructive cyclic testing, 18 reamed femur specimens were subjected to failure loads in four-point bending and proximal axial compression. There were four testing groups representing 13, 14, 15 mm IM reaming sizes (most commonly used for bone graft harvesting), and

19 mm IM reaming size (the most extreme example of a reamed femur). The specimens were loaded to failure in four-point medial bending (with the lateral aspect in tension) with the same testing setup described for four-point bending stiffness testing (Figure 1C) at a rate of 10 mm/min in displacement control. The remaining proximal portions of each femur were separately loaded to failure to study the effects of IM reaming on the entry portal. These femurs were osteotomized 12 cm distal to the greater trochanter (proximal to the failed diaphyseal region), and mounted in a custom potting clamp molded from PMMA to allow loading along the mechanical axis. The construct was rigidly fixed to the MTS machine, and the femoral head was loaded through a rail bearing at a rate of 10 mm/min until complete fracture (Figure 1D). The peak load at failure and cross-head displacement were recorded as well as the mode/type of fracture resulting for each failure test.

Statistical Analysis

The data from all testing modes were tabulated and analyzed. Load versus displacement curves were generated from the 10th cycle of each cyclic test, and stiffness was determined as the slope of elastic region of the curve. Stiffness and peak failure load were calculated for each destructive test. Statistical analyses of cyclic and destructive testing results were performed using analysis of variance (ANOVA) with Bonferroni adjusted alphas (SAS Institute; Cary, NC, USA). Linear regression was used to assess the whole bone cyclic testing data and determine the correlation between the changes in femur stiffness and the IM reaming diameter. The mid-diaphysis and proximal destructive tests data were compared with paired t-tests.

RESULTS

Cyclic Nondestructive Testing

The mean stiffness values for whole bone axial testing are depicted in Figure 2. Statistically significant decreases in stiffness were observed in the 16, 17, 18, and 19 mm

groups compared with the intact controls, and stiffness decreased linearly as the reaming diameter increased (Figure 2). Linear regression analysis demonstrated that whole bone axial compression stiffness changed by -0.053 N/mm for every 1-mm increase in reaming size, with a 95%

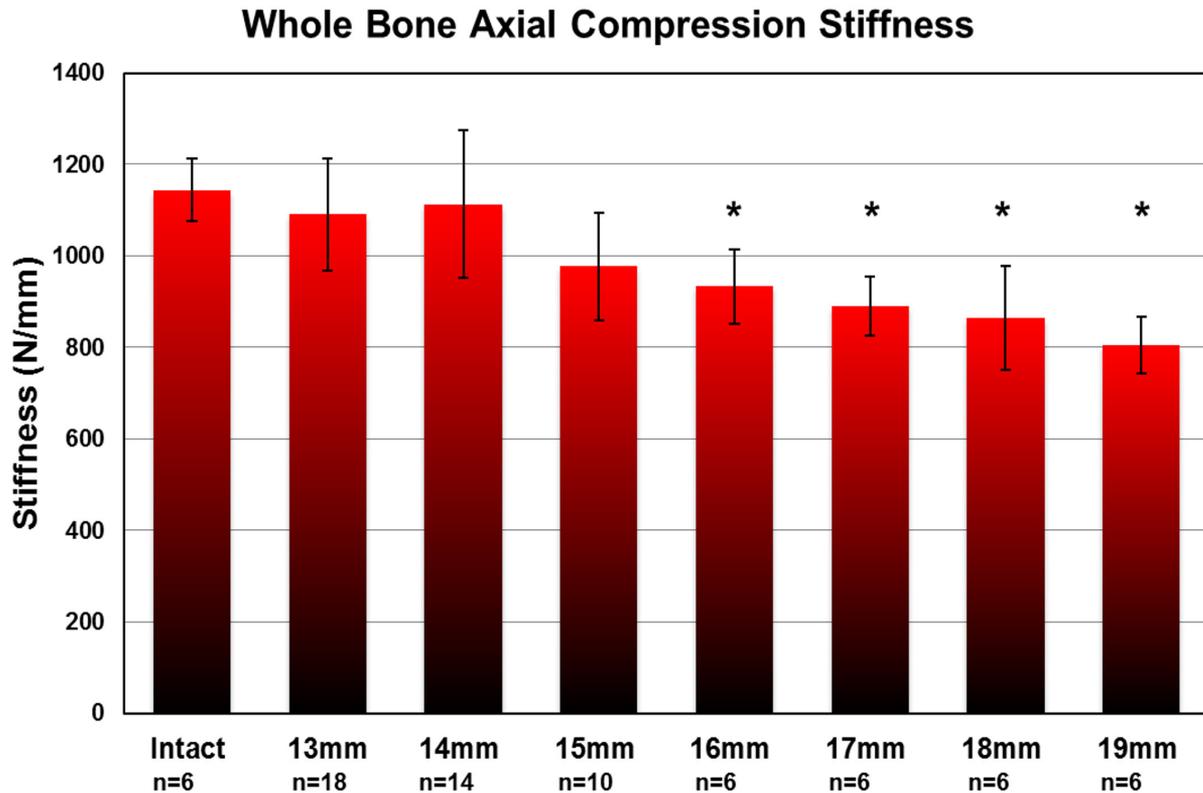


Figure 2. The average whole bone axial compression stiffness at each sequential reaming level from 13 mm to 19 mm diameter (* denotes statistically significant p-value compared with intact controls based on an adjusted alpha of 0.007 (0.05/7)).

confidence interval spanning -0.06717 and -0.03875 N/mm, a corresponding significance of $p < 0.0001$ and $r^2 = 0.4549$.

The mean stiffness values for non-destructive whole bone cyclic torsion testing are depicted in Figure 3. Torsion testing demonstrated no significant difference in mean stiffness between external and internal rotation (Figure 3). With the exception of the 17-mm specimens, there was a statistically significant decrease in stiffness from

the intact specimens as the IM reaming diameter increased from 15 to 19 mm. There was a weak linear relationship ($r^2 = 0.0461$) for stiffness and reaming size for whole bone cyclic torsion testing (Figure 3). Linear regression analysis demonstrated that for every 1-mm increase in the size of IM reaming, stiffness decreased 0.0076 N/mm, with a 95% confidence interval spanning -0.0135 and -0.0017 N/mm with a corresponding significance of $p = 0.0134$.

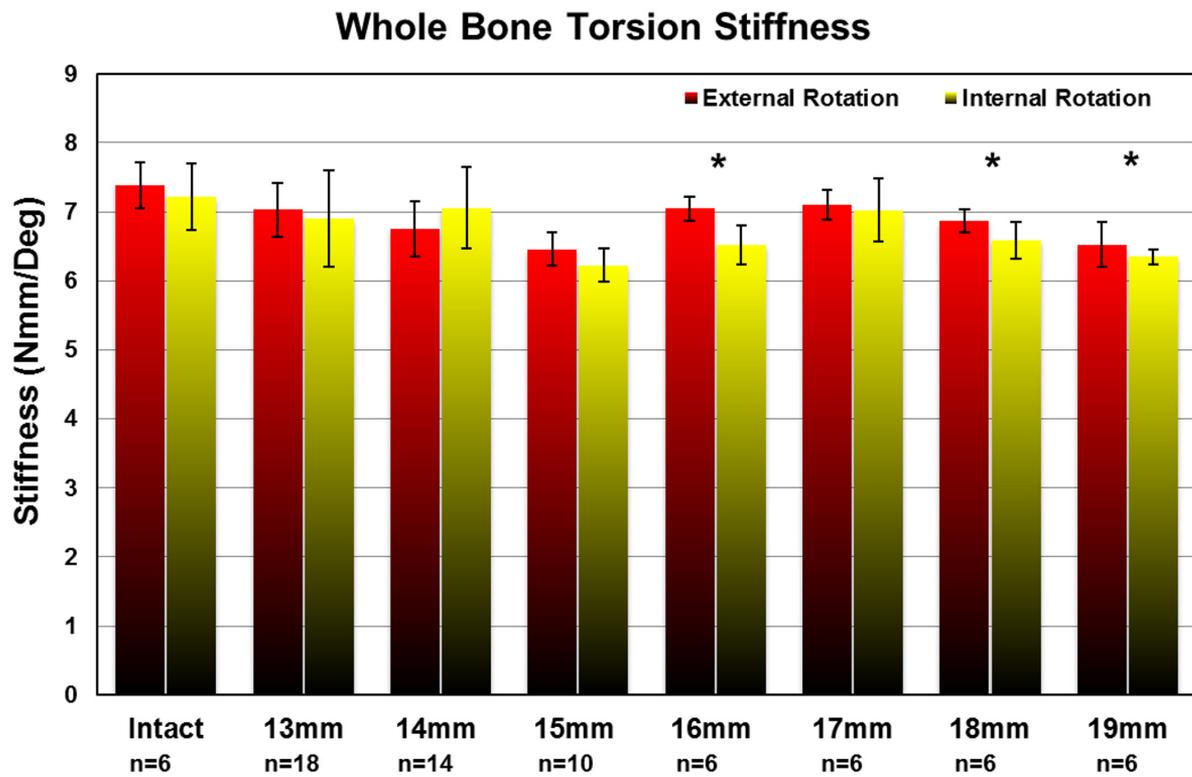


Figure 3. The average whole bone torsional stiffness at each sequential reaming from 13 mm to 19 mm. Results are shown for both external and internal rotation (* denotes statistically significant p-value compared with intact controls).

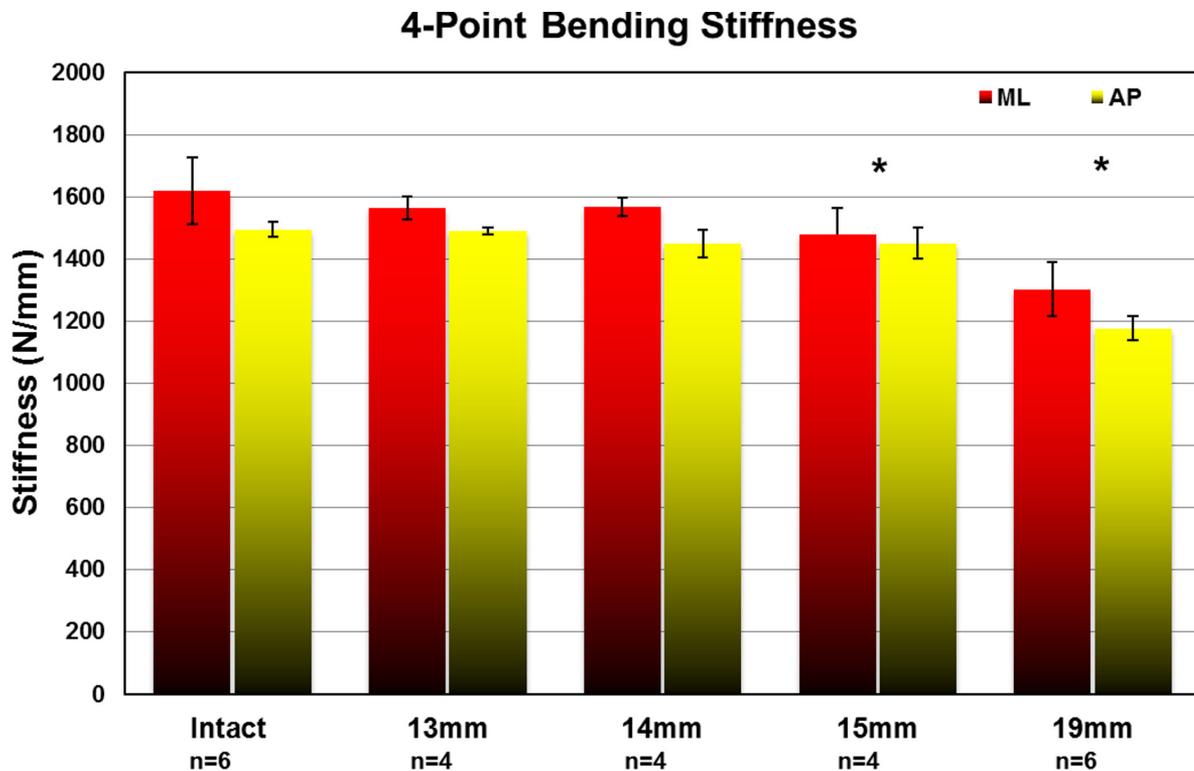


Figure 4. The average four-point bending stiffness for each reaming diameter (* denotes statistically significant difference). Results are shown for both, medial-lateral (ML) and anterior-posterior (AP) planes.

Nondestructive cyclic testing mean stiffness values for four-point bending are shown in Figure 4. IM reaming diameters of 15 and 19 mm showed statistically significant decreases in stiffness compared with intact specimens in both medial bending and posterior bending planes. While there was a statistically significant ($p < 0.0001$) decrease in stiffness with posterior versus medial four-point bending, there was no correlation between the size of IM reaming and the testing plane (Figure 4).

Load to Failure Testing

Mean load to failure for the destructive four-point mid-diaphyseal bending tests and proximal femur axial compression tests are

illustrated in Figure 5. IM reaming to 19 mm resulted in statistically significant reduction in load to failure for both mid-diaphyseal bending and proximal femur axial testing modes compared with the 13-mm reaming diameter. Load to failure of the proximal femur was significantly lower ($p = 0.0037$) than that of the mid-diaphysis. Furthermore, there was no statistically significant difference in stiffness in four-point bending or proximal fracture testing for any (13, 14, 15, and 19 mm) reaming size. Fracture patterns of the proximal femur as a result of axial compressive loading for each reaming diameter are depicted in Figure 6. All fractures consistently propagated through the trochanteric reaming entry site.

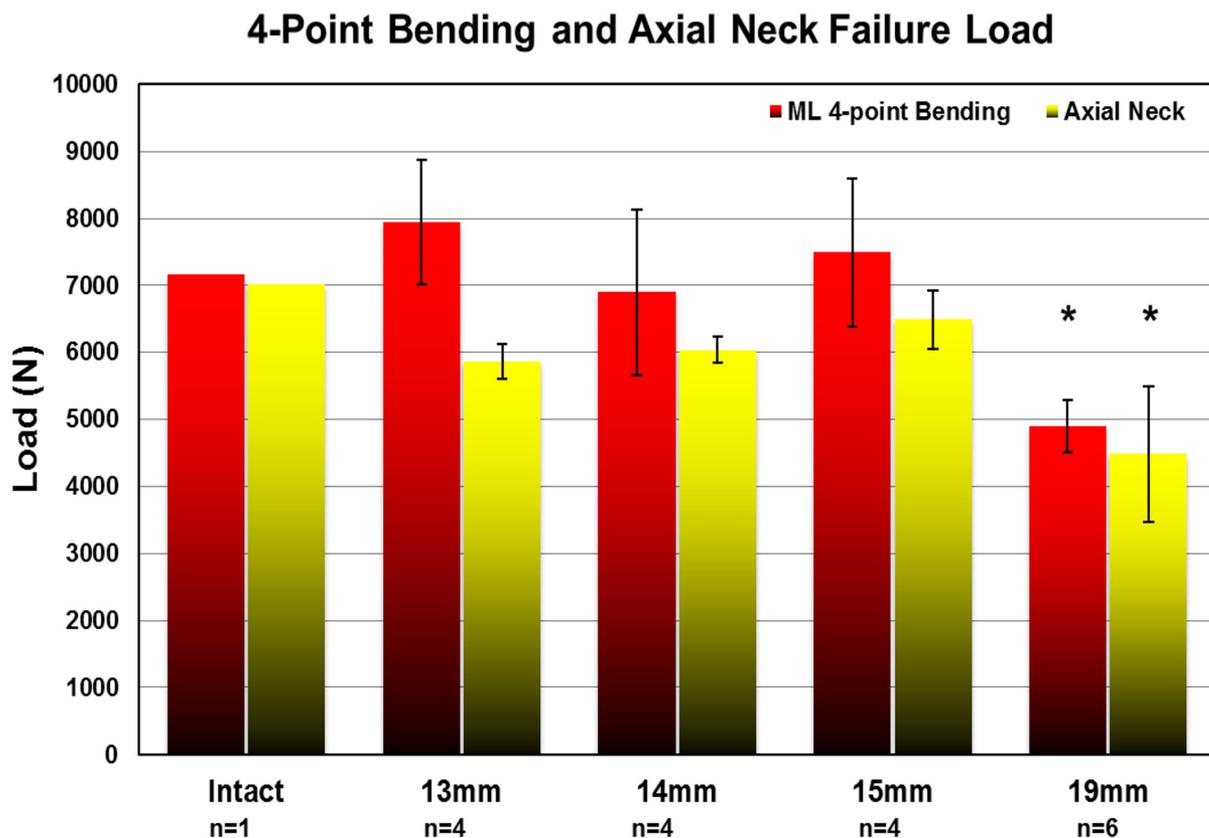


Figure 5. The average medial-lateral (ML) mid-diaphyseal four-point bending failure load and average proximal femur compression failure load for each sequential reaming diameter ranging from 13 mm to 19 mm diameter (* denotes statistically significant p-value compared with intact control).

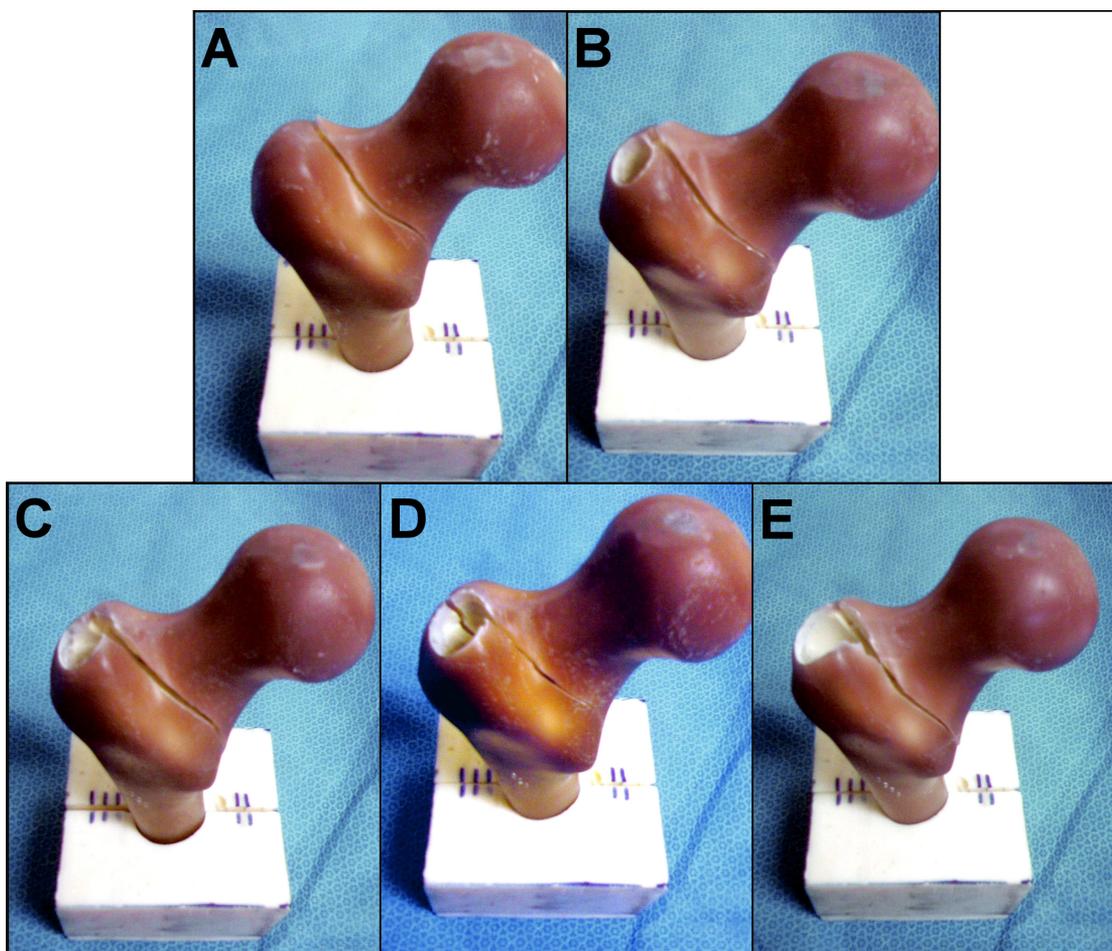


Figure 6. Fracture patterns resulting from axial compressive failure of the proximal femur for each reaming level: the intact femur (A); reamed at 13 mm (B); 14 mm (C); 15 mm (D); and 19 mm (E) IM diameters.

DISCUSSION

Presently, several generally accepted indications exist for femoral IM reaming without subsequent nail placement. Among these are IM canal debridement in the treatment of infection, and the enhancement/acceleration of delayed bone healing without re-implantation [5]. Perhaps the most frequent indication for femur IM reaming without nail fixation is the use of the reamer-irrigator-aspirator (RIA) system [9] as a means of autogenous bone graft harvesting [7,8]. In this clinical setting, a significant amount of bone graft may be obtained from the femoral endosteal surface, and increasing

the amount of bone graft harvested can be achieved by simply increasing the size of the IM reamer head. Regardless of the indication for IM reaming without nail placement, the effects of reaming on the mechanical properties of the residual bone strength become a reasonable concern.

Entry-site fractures following reamed femoral IM nail fixation have been reported by several authors [12,13] with incidence as high as 3% [14], and were attributed to the location and size of the IM nail entry site [15,16]. Interestingly, Küntscher [17] recommended the tip of the greater trochanter as the optimal entry site in his original description of the

femoral IM nailing technique. However, the greater trochanter is located lateral to the central IM canal axis and straight IM nails inserted through this entry site tended to impinge on the medial cortex. A more medial piriformis fossa entry has been proposed to address this issue [14], and although this entry is more favorable, this approach can cause significant damage to hip muscle and tendons as well as to the femoral head blood supply [18]. However, since the advent of flexible IM reamers and slightly curved IM nails, the tip of the greater trochanter has regained its popularity as an entry site for reamed antegrade femoral IM nailing. Ricci et al. [19] demonstrated that greater trochanteric IM nailing has considerable clinical merit to include reduced surgical fluoroscopy time and decreased overall operative time, especially in obese patients.

In a cadaveric study, Strand et al. [20] demonstrated the biomechanical effects of femoral IM reaming entry sites (ie, the piriformis fossa versus the tip of the greater trochanter) on the biomechanical integrity of the proximal femur. In their study, a substantial weakening of the proximal femur was observed with entry through the piriformis fossa compared with the greater trochanter (23.2% vs. 11.2% strength-to-failure and 45% vs. 11% energy-to-failure reductions of the intact control, respectively). Because proximal femur fractures post IM nailing occurred more consistently through the piriformis fossa entry site, the authors concluded that clinically significant weakening of the proximal femur was less likely to occur through the trochanteric entry site.

In a drilled-hole entry site model without IM canal reaming study, Miller et al. [3] demonstrated that a well-positioned

hole in the piriformis fossa exhibited minimal effects on the residual stiffness and strength of the proximal femur, irrespective of a 10-mm versus a 14-mm hole diameter. These authors, however, also determined that deviation from the ideal piriformis fossa entry site can result in greater weakening of the proximal femur than that simply due to the size of the entry hole. Interestingly, all of the experimental specimens in this study fractured through the drilled entry hole, whereas all of the control intact femurs fractured medially at the base of the femoral neck.

Finnan et al. [21] performed a cadaveric femur study using RIA, a system for IM bone graft harvesting, and determined that the biomechanical effects of the 15-mm RIA reamer were not statistically significant regardless of the entry site (antegrade piriformis fossa or greater trochanteric, or retrograde distal femur entry site) that was used. After the manufacturer recommended single-pass RIA reaming of the IM canal, all specimens withstood single-leg stance cyclic loading, and exhibited no apparent differences in load to failure. These authors concluded that RIA did not significantly diminish the biomechanical properties of the femur, and that it can be safely applied in a clinical setting without protected weightbearing [21].

In the present study, sequential IM reaming with increasing reamer diameters without nail fixation was performed to assess the effects of IM reaming on residual long bone stiffness, strength, and failure in composite femurs subjected to simulated physiological loads. The results demonstrated a statistically significant linear decrease in stiffness in whole bone axial compression with IM reaming to consecutively larger diameters. Similar results, although less

linear, were observed for whole bone tensional stiffness. Four-point mid-diaphyseal bending exhibited a significant decrease in stiffness for higher reaming sizes, while load to failure was significantly reduced only for IM reaming to a diameter of 19 mm. Furthermore, the load to failure and the mode of failure at the proximal femur accentuated the importance of the size of the antegrade femur IM reaming entry site. The consistent fracturing (failure) that occurred in continuity with the trochanteric entry site and the progressive lateralization of the fracture pattern with increased reaming size suggest that the proximal femur antegrade IM reaming entry is a significant stress riser. The significant reduction in the failure loads that culminated in these fracture patterns further affirms the susceptibility of the proximal femur to fracture when subjected to IM reaming without subsequent nail fixation.

This study demonstrates that the IM reamed diaphysis of the femur retains a significant portion of its mechanical properties, even when subjected to reaming diameters that are not typically employed in the clinical setting. The largest reaming diameter (19 mm) created a statistically significant decrease in four-point bending load to failure as compared with the smaller, more commonly utilized reaming sizes (13 to 15 mm diameters). The 15-mm reamed specimens retained, on average, 94.34% of their initial strength, while the 19-mm reamed specimens retained only 61.73%. In all reamed femur specimens, the neck-shaft junction appears to be the weakest portion of the femur.

There are both limitations and advantages of having used a composite femur model in this biomechanical study. First, the use of a single-size femur obviates the

need for testing data adjustments due to the variability in femur size and strength encountered in a normal patient population. However, the use of composite, standardized femurs excluded the inter-specimen variability in size, mineral density, and geometry that is prevalent in cadaveric specimens. Furthermore, the use of cadaveric femurs may not accurately represent the long bone biomechanics inherent in the patient population for which femoral IM reaming without subsequent nail fixation is typically applied. The age-related reduction of biomechanical properties of typical elderly cadaveric specimens is well appreciated [22]. The clinical use of IM reaming without a nail as a method of autograft harvesting in the elderly is limited because of concerns about poor bone stock and the reduced osteogenic potential of IM marrow and bone autograft. Currently, the clinical indications for the RIA system have been primarily limited to young patients with good bone stock. Moreover, the limited availability of cadaveric femurs from young donors would be prohibitive owing to difficulties associated with obtaining an adequate sample size.

CONCLUSIONS

Reaming of the IM canal without nail fixation in a composite femur is associated with a small reduction of bone stiffness and strength at smaller reamer diameters (13-16 mm); however, this reduction becomes significant at larger reamer diameters (17-19 mm). The trochanteric entry site for IM reaming makes the proximal femur susceptible to a trochanteric fracture, and this risk increases with the reaming diameter.

REFERENCES

- [1] Bong MR, Kummer FJ, Koval KJ, Egol KA. Intramedullary nailing of the lower extremity: biomechanics and biology. *J Am Acad Orthop Surg.* 2007;15: 97-106.
- [2] Fairbank AC, Thomas D, Cunningham B, Curtis M, Jinnah RH. Stability of reamed and unreamed intramedullary tibial nails: A biomechanical study. *Injury.* 1995;26:483-5.
- [3] Miller SD, Burkart B, Damson E, Shrive N, Bray RC. The effect of the entry hole for an intramedullary nail on the strength of the proximal femur. *J Bone Joint Surg Br.* 1993;75:202-6.
- [4] Zdero R, Bougherara H, Dubov A, Shah S, Zalzal P, Mahfud A, Schemitsch EH. The effect of cortex thickness on intact femur biomechanics: a comparison of finite element analysis with synthetic femurs. *Proc Inst Mech Eng H.* 2010;224(H7):831-40.
- [5] Bellapianta J, Gerdeman A, Sharan A, Lozman J. Use of the reamer irrigator aspirator for the treatment of a 20-year recurrent osteomyelitis of a healed femur fracture. *J Orthop Trauma.* 2007;21:343-6.
- [6] Kobbe P, Tarkin IS, Pape HC. Use of the 'reamer irrigator aspirator' system for non-infected tibial non-union after failed iliac crest grafting. *Injury.* 2008;39:796-800.
- [7] Frölke JP, Nulend JK, Semeins CM, Bakker FC, Patka P, Haarman HJ. Viable osteoblastic potential of cortical reamings from intramedullary nailing. *J Orthop Res.* 2004;22:1271-5.
- [8] Van Gorp CC, Falk JV, Kmiec SJ, Siston RA. The reamer/irrigator/aspirator reduces femoral canal pressure in simulated TKA. *Clin Orthop Relat Res.* 2009;467:805-9.
- [9] Synthes. Reamer/Irrigator/Aspirator (RIA) for intramedullary reaming and bone harvesting. Technique guide. http://www.synthes.com/sites/NA/Products/Trauma/IntramedullaryNailingSystems/Pages/Reamer_Irrigator_Aspirator_RIA.aspx Accessed Jul 5, 2015.
- [10] Heiner AD. Structural properties of fourth-generation composite femurs and tibias. *J Biomech.* 2008; 41: 3282-4.
- [11] Gardner M, Chong A, Pollock AG, Woolley PH. Mechanical evaluation of large-size fourth-generation composite femur and tibia models. *Ann Biomed Eng.* 2010;38:613-20.
- [12] Böstman O, Varjonen L, Vainionpää S, Majola A, Rokkanen P. Incidence of local complications after intramedullary nailing and after plate fixation of femoral shaft fractures. *J Trauma.* 1989;29:639-45.
- [13] Brumback RJ, Reilly JP, Poka A, Lakatos RP, Bathon GH, Burgess AR. Intramedullary nailing of femoral shaft fractures. Part I. Decision-making errors with interlocking fixation. *J Bone Joint Surg Am.* 1988;70:1441-52.
- [14] Brumback RJ, Uwagie-Ero S, Lakatos RP, Poka A, Bathon GH, Burgess AR. Intramedullary nailing of femoral shaft fractures. Part II. Fracture healing with static interlocking fixation. *J Bone Joint Surg Am.* 1988;70:1453-62.
- [15] Christie J, Court-Brown C, Kinninmonth AW, Howie CR. Intramedullary locking nails

in the management of femoral shaft fractures. *J Bone Joint Sur Br.* 1988;70:206-10.

[16] Hooper GJ, Lyon DW. Closed unlocked nailing for comminuted femoral fractures. *J Bone Joint Surg Br.* 1988;70:619-21.

[17] Bick EM. The classic: The intramedullary nailing of fractures by G. Küntscher. *Clin Orthop Relat Res.* 1968;60:5-12.

[18] Dora C, Leunig M, Beck M, Rothenfluh, D, Ganz R. Entry point soft tissue damage in antegrade femoral nailing: a cadaver study. *J Orthop Trauma.* 2001;15:488-93.

[19] Ricci WM, Schwappach J, Tucker M, Coupe K, Brandt A, Sanders R, Leighton R. Trochanteric versus piriformis entry portal for the treatment of femoral shaft fractures. *J Orthop Trauma.* 2006;20:663-7.

[20] Strand RM, Mølster AO, Engesaeter LB, Gjerdet NR, Orner T. Mechanical effects of different localization of the point of entry in femoral nailing. *Arch Orthop Trauma Surg.* 1988;117:35-8.

[21] Finnan RP, Prayson MJ, Goswami T, Miller D. Use of the Reamer-Irrigator-Aspirator for bone graft harvest: a mechanical comparison of three starting points in cadaveric femurs. *J Orthop Trauma.* 2010;24:36-41.

[22] McCalden RW, McGeough J.A, Court-Brown CM. Age-related changes in the compressive strength of cancellous bone. The relative importance of changes in density and trabecular architecture. *J Bone Joint Surg Am.* 1997;79:421-7.