Influence of Gender and Fixation Stability on Bone Defect Healing in Middle-aged Rats: A Pilot Study

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Abstract

Background Gender and stability of fixation independently influence bone regeneration but their combined effects are unclear.

Questions/purposes In a pilot study we determined the combined influence of gender and fixation stability on the callus of middle-aged rats regarding (1) biomechanical properties; (2) bridging over time; (3) callus formation; and (4) callus size, geometry, mineralization, and microstructure.

Methods We osteotomized the left femur of 32 Sprague-Dawley rats (12 months old). Femurs were externally fixed with a gap of 1.5 mm in four groups of eight animals each: female semirigid, male semirigid, female rigid, and male rigid. Qualitative and quantitative in vivo radiographic analyses were performed twice weekly. Six weeks

postoperatively, harvested femora were evaluated using micro-CT and biomechanical testing.

Results Torsional stiffness and maximum torque at failure were higher in male and in semirigidly fixed fractures. Radiographic analysis revealed earlier bridging and callus formation in both male groups. Micro-CT analysis showed a larger callus size, altered geometry, and microstructure in males and semirigidly fixed animals, whereas mineralization was similar in all animals.

Conclusion Our data suggest female gender represents an independent risk factor for bone healing in middle-aged rats. Although healing in females was delayed compared with males, they exhibited a similar response (superior callus properties) to a more semirigid fixation.

Clinical Relevance While female gender appears to reflect a risk for impaired bone healing in middle-aged female rats, clinical studies would be required to confirm the finding in humans.

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Introduction

Despite the current standards in internal fixation of human fractures and bone defects, delayed healing or nonunions occur with a frequency of 5% to 20%, resulting in high costs for the healthcare system [8, 21, 28]. Both biologic and mechanical factors affect the outcome and course of bone healing [10, 12–15, 19, 24]. In addition to perfusion, systemic metabolism, and age, previous animal and large clinical studies have identified female individuals to be at risk for impaired bone healing [2, 5, 11, 16, 22].

The local mechanical environment also influences healing outcome [1, 12, 15, 19, 27]. Initial instability can delay bone healing or lead to nonunions as a result of rupturing of the bridging tissue, including the nutrimental

blood vessels [10, 14, 15, 17]. On the other hand, if mechanical stimuli are completely absent, bone healing may also be delayed [6, 10].

Clinically, gender and stability affect bone defect healing simultaneously. It is unclear whether gender and stability interact in some synergistic or deleterious way. Knowledge of synergistic or independent effects of these factors might suggest a gender-related modification in the stability of clinical fracture devices that could possibly improve bone healing outcome.

We recently reported that the healing outcome using different fixation stabilities is dependent on the individual's age [24]. Rigid external fixation led to improved mechanical properties of the callus and to higher bridging in a group of young compared with middle-aged mature female rats. Because impaired bone healing occurs more frequently in adult or aged individuals [7], we further investigated bone healing in middle-aged rats. We observed superior mechanical callus properties, a larger callus size, earlier bridging, and a higher mineralization and polar moment of inertia [Jz] in microcomputed tomography (μ CT) in middle-aged male compared with female rats, both stabilized with rigid fixation [22].

The purpose of the present pilot study was to explore the combined influence of gender and fixation stability on bone healing in a middle-aged small animal model and to create research questions regarding the effects on: (1) the biomechanical properties of the callus of middle-aged rats after 6 weeks; (2) the radiographic bridging of the artificial bone defect over time; (3) the increase in radiographic callus size over time; and (4) the callus mineralization, geometry, size, and microstructure at 6 weeks followup.

Material and Methods

To explore the independent or dependent influence of gender and fixation stability on the dependent variables, we created four groups of eight 12-month-old Sprague-Dawley rats each (Fig. 1). In groups MS and FS, a semirigid configuration of the fixator was chosen, whereas in groups MR and FR, a rigid fixator was used as previously reported [23]. Recently published (maximum torque at failure [MTF], torsional stiffness [TS], qualitative radiographic score, Jz, cross-sectional area [CsAr]) results of the female aged groups of our first study [24] and of the semirigidly fixated aged male group of the second study [22] served as historical controls for comparison (all data published earlier are marked within the tables and figures). All experiments were carried out according to the policies and principles established by the Animal Welfare Act, the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the National Animal Welfare

Fig. 1 The figure shows the numbers of animals included and their group distribution regarding the independent variables gender and fixation stability. Variable category biomechanical properties refer to dependent variables maximum torque at failure (MTF), torsional stiffness (TS), and energy to failure (E_{failure}). Mineralization is reflected by callus tissue mineral density (TMD) and callus mineral density (BMD), size by callus volume (BV) and callus cross-sectional area (CsAr), geometry by polar moment of inertia (Jz), and microstructure by callus strut number (Tb.N), spacing (Tb.Sp), and thickness (Tb.Th). Callus formation (3) is illustrated by change of relative callus cross-sectional area (dCSA). Superscript letters (a) [22] and (b) [24] indicate methods, in which historical controls of recently published parameters were used for statistical comparison.

Guidelines and were approved by the local legal representative (Landesamt für Gesundheit und Soziales Berlin: G 0190/05 and G 0071/07). Because this study is a preliminary pilot study, which is believed to generate new hypotheses, no a priori power analysis was performed.

Operations were performed by one surgeon (PS) under aseptic conditions on a heating plate (37°C) under general anesthesia according to previously published protocols [9, 24], which are briefly summarized here. The midshaft left femur was prepared using an anterolateral approach between hamstring muscles and M. quadriceps femoris. Four threaded 1.2-mm Kirschner wires were implanted after drilling. The fixed femur was osteotomized (drill: 0.8 mm diameter, S-11; saw: 8R; Implantmed[®]; W&H Oral Surgery, Bürmoos, Austria), distracted to a gap of 1.5 mm, and finally fixed with the definitive fixator by defining the group-specific offset using a precision spacer. A previously defined external rat femoral fixator was used to establish standardized mechanical differences between the groups [23]. The rigid setup (7.5-mm offset) resulted in an axial stiffness of 25.2 ± 2.6 N/mm and in a TS of

Table 1. Animal weights (g)

Time		Surgery	Euthanasia
Group	MR	366 ± 13	389 ± 15
	MS	363 ± 9	378 ± 19
	FR	333 ± 27	357 ± 32
	FS	352 ± 40	365 ± 38
Gender	p*	0.0299	0.0390
Fixation	p*	0.4111	0.8859
Gender × fixation	p*	0.2611	0.3671

* p Values are from two-way analysis of variance. Post hoc tests were not performed because combined effects of gender × fixation were not significant; values are reported as mean ± SD.

8.1 ± 1.2 Nmm/°, whereas the axial stiffness was 10.4 ± 2.2 N/mm and TS was 6.6 ± 1.2 Nmm/° in the semirigid setup (15-mm offset) [23].

Postoperatively animals were housed individually and allowed unrestricted weightbearing. All were checked daily and analgesia (buprenorphine 10% subcutaneously; Essex Pharma GmbH, Munich, Germany) was given if the animals presented signs of pain (no weightbearing, lack of grooming, or cowering posture). No antibiotics were administered. Postoperatively, rectal temperature and body weight (Table 1) were measured weekly to detect possible infections (temperature 38°C or greater; weight loss greater than 10% of body weight over 1 week).

Immediately after surgery and every 2 weeks thereafter until euthanasia, we obtained two standardized radiographic views (AP, lateral, 30 cm distance to the film; Mobilett Plus X-Ray unit; Siemens, Erlangen, Germany, films: Chronex 5 Medical X-Ray Film; AGFA-Gevaert NV, Mortsel, Belgium). Radiographic examinations were performed while the animals were administered inhalation anesthesia (isoflurane; Baxter Deutschland GmbH, Unterschleissheim, Germany; mixed with oxygen and N₂O).

Six weeks postoperatively, all animals were euthanized by intracardiac injection of 5 mL potassium chloride (7.45%; Braun Melsungen AG, Melsungen, Germany) while under deep inhalation anesthesia (see previously). After the animals were euthanized, the osteotomized and contralateral femora were harvested.

Apparent pseudarthroses observed clinically or radiographically were noted after collecting the bones and excluded from biomechanical testing as a result of the immature callus tissue. Pseudarthroses were defined as a radiographic score of 3 (as described subsequently) at euthanasia and/or an interfragmentary gap that macroscopically contained only fibrotic tissue and showed low mechanical stability after removing the fixator [22]. One male animal (group MS) was excluded from further analysis as a result of infection. An additional male animal from group MR presented a broken Kirschner wire at

4 weeks followup and was also excluded. In 13.3% of the animals, a pseudarthrosis was recorded. Pseudarthrosis was found in four animals (MR one; FS one; FR two) but without a difference ($p = 0.566$) in incidence between the groups. No other clinical or implant-associated complications were observed during followup.

After precalibration, the harvested bones were scanned using a Viva40 micro-CT (Scanco Medical AG®, Brüttisellen, Switzerland) at a voltage 70 kVp, current of 114 µA, and a fixed isotropic resolution of 12.5 µm. The scan axis coincided nominally with the diaphyseal axis of the femur. Because of a data storage failure during scanning, three animals in the MS and MR groups could not be included in µCT analysis. On each two-dimensional tomogram, the cortical bone was masked out using a manually drawn contour. The resulting gray-scale images of the whole callus were segmented using a fixed global threshold of 550 mg HA/cm², rendering a purely mineralized callus, while excluding all cortical bone.

Immediately after µCT scanning (a maximum of 3 hours after euthanasia), both femora of each animal were mechanically tested to determine MTF, TS, and energy to failure (E_{failure}) of the operated femur, as previously described [20, 24]. All three parameters were reported as a percentage of the values from the intact contralateral side [26] and statistically evaluated for the influence of gender (male/female) and fixation stability (rigid/semi-rigid) by a two-sided two-way analysis of variance (ANOVA) after successfully testing for normality of distribution (Kolmogorov-Smirnov test). Intergroup comparison was performed by post hoc Bonferroni tests only if a statistical interaction (other than separate influence) between the influence of gender and fixation stability was observed.

To determine bridging of the bone defects over time, two independent observers (PS, MM) performed blinded qualitative radiographic analysis using the following criteria in two perpendicular views for each case and time point while hiding fixation configuration [20]: (1) complete bridging (four cortices bridged by callus); (2) incomplete bridging (one to three cortices bridged by callus); and (3) no bridging (no cortex bridged by callus). In case of divergent scoring, a decision was made by a third independent observer (orthopaedic surgeon CP). Interobserver variability was tested by kappa statistics (kappa = 0.86). Bridging scores were statistically screened for differences between the groups by a chi-square test at each followup. If the differences were significant, we performed intergroup comparisons using Fisher's exact test.

To determine differences in callus size over time, radiographs were scanned, normalized, and scaled using the Kirschner wire diameter. The cross-sectional area of each callus (CSA) was measured and expressed relative to

that of the corresponding femur itself (in percentage) [24]. Intraindividual changes of CSA among Weeks 0 to 2 ($dCSA_{0-2}$), 2 to 4 ($dCSA_{2-4}$), and 4 to 6 ($dCSA_{4-6}$) were compared between the groups using a two-way ANOVA as described for mechanical testing parameters.

From the microcomputed binarized images, we made the following morphometric measurements using a direct three-dimensional approach with the vendor software (Scanco software V.6; Scanco Medical AG[®], Brüttisellen, Switzerland): (1) callus mineral density (BMD [mg HA/cm²]); (2) callus tissue mineral density (TMD [mg HA/cm³]) for determination of callus mineralization; (3) polar moment of inertia (Jz [mm⁴]) for determination of callus geometry; (4) callus volume (BV [mm³]); (5) callus cross-sectional area (CsAr [mm²]) for determination of callus size; (6) callus strut number (Tb.N [1/mm]); (7) callus strut thickness (Tb.Th [mm]); and (8) callus strut spacing (Tb.Sp [mm]) for determination of callus microstructure. All these dependent parameters were statistically evaluated by two-way ANOVA.

The statistical analysis was performed using statistics software, SPSS 17.0 (SPSS Inc, Chicago, IL) and Prism 5.0 (GraphPad Software Inc, San Diego, CA).

Results

After 6 weeks, the mean TS of the operated, in relation to the contralateral, femur was higher ($p = 0.004$) in male versus female animals and similar ($p = 0.164$) in semirigid versus rigid fixation (Fig. 2). At the same time, MTF of the bones in relation to that of the contralateral femur was higher ($p < 0.001$) in male versus female animals and

Fig. 2 The illustration shows torsional stiffness of the callus (TS) relative to that of the contralateral femur between the groups at 6 weeks. Because we found no interaction (gender $\times$ fixation $p = 0.757$) between influencing parameters gender and fixation stability, no intergroup comparison was performed. Whiskers indicate single SD. Superscript letters (a) [24] and (b) [22] indicate that the values have been recently published and were used as historical controls for statistical comparison.

higher ($p < 0.001$) in semirigid versus rigid fixation (Fig. 3). $E_{failure}$ was higher ($p = 0.006$) in animals with semirigid compared those with rigid fixation (Fig. 4).

In all groups, progressive bony bridging could be observed in radiographic scores over time (Table 2). At 2-week followup, bony bridging was highest ($p = 0.008$) in MS followed in order by MR, FS, and FR. Cortices were bridged more often ($p = 0.001$) in male (MR + MS) versus female (FR + FS) animals.

Between surgery and 2-week followup, male animals presented a higher ($p = 0.004$) mean increase in relative cross-sectional area of the callus ($dCSA_{0-2}$) than female animals. Additionally, $dCSA_{0-2}$ was larger ($p < 0.001$) in semirigid than in rigid fixation groups. Besides the separate effects, we observed a combined effect ($p < 0.001$) of

Fig. 3 The figure shows maximum torsional moment at failure of the callus (MTF) relative to the contralateral femur between the groups at 6 weeks. Because we found no combined influence (gender $\times$ fixation $p = 0.865$) of the parameters gender and fixation stability, no intergroup comparison was performed. Whiskers indicate single SD. Superscript letters (a) [24] and (b) [22] indicate that the values have been recently published and were used as historical controls for statistical comparison.

Fig. 4 The figure shows energy to failure of the callus ($E_{failure}$) relative to the contralateral femur between the groups at 6 weeks. Because we found no combined influence (gender $\times$ fixation $p = 0.712$) of the parameters gender and fixation stability, no intergroup comparison was performed. Whiskers indicate single SD.

Table 2. Radiographic score

Time postoperatively	Score (bridging)	Number of animals			
		MS ^b	MR	FS ^{a,b}	FR ^a
2 weeks	1 (complete)	0	0	0	0
	2 (incomplete)	5	4	1	0
	3 (no bridging)	2	3	7	8
	p*	0.008			
4 weeks	1 (complete)	3	1	0	1
	2 (incomplete)	4	4	5	4
	3 (no bridging)	0	2	3	3
	p*	0.320			
6 weeks	1 (complete)	4	2	1	1
	2 (incomplete)	3	4	6	6
	3 (no bridging)	0	1	1	1
	p*	0.490			

* p Values from chi-square test for differences among all four groups at the followup. At 2 weeks, intergroup comparison showed a better scoring (Fisher's exact test) of MR > FR ($p = 0.007$) and MR > FS ($p = 0.041$) as well as of MS > FR ($p = 0.026$). At 4 and 6 weeks, intergroup comparison also revealed no statistical significance between the groups. Superscript letters (a) [24] and (b) [22] indicate that the values have been recently published and were used as historical controls for statistical comparison.

Fig. 5 The figure shows the change in relative cross-sectional area (dCSA) between groups over time intervals. Whiskers represent single SD. Because no combined effect of gender and fixation stability (gender $\times$ fixation $p = 0.077$) was observed between Weeks 2 and 4, no intergroup comparison was performed. Between Weeks 4 and 6, we found no differences in intergroup comparisons in post hoc tests. p Values are from post hoc Bonferroni tests.

Table 3. In vitro three-dimensional micro-CT metrics of the geometry, size, and mineralization of the callus at 6 weeks

Factor	Groups	BV (mm ³)	Jz (mm ⁴)	CsAr (mm ²)	TMD (mg HA/cm ³)	BMD (mg HA/cm ³)
Groups	MR	22 $\pm$ 10	51 $\pm$ 37	9 $\pm$ 4	920 $\pm$ 27	213 $\pm$ 61
	MS	44 $\pm$ 9	102 $\pm$ 28 ^a	16 $\pm$ 3 ^a	927 $\pm$ 43	269 $\pm$ 50
	FR	11 $\pm$ 6	13 $\pm$ 8	4 $\pm$ 2	929 $\pm$ 42	89 $\pm$ 42
	FS	18 $\pm$ 7	29 $\pm$ 18 ^a	6 $\pm$ 2 ^a	916 $\pm$ 22	138 $\pm$ 51
Gender	p*	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.950$	$p < 0.001$
Fixation	p*	$p < 0.001$	$p = 0.001$	$p = 0.001$	$p = 0.859$	$p = 0.019$
Gender $\times$ fixation	p*	$p = 0.021^{\dagger}$	$p = 0.069$	$p = 0.095$	$p = 0.484$	$p = 0.858$

All parameters are presented as mean $\pm$ single SD; * p values from two-way analysis of variance; [†] post hoc tests and intergroup comparison for BV was performed (Fig. 7) because combined effects of gender and stability was significant regarding this parameter; BV = callus volume; Jz = polar moment of inertia; CsAr = callus cross-sectional area; TMD = callus tissue mineral density; BMD = callus mineral density; superscript letter (a) indicates that the values have been recently published [22] and were used as historical controls for statistical comparison.

gender and fixation stability variation (Fig. 5). Between 2- and 4-week followup, dCSA₂₋₄ was higher ($p = 0.005$) in male compared with female animals, whereas no difference ($p = 0.666$) in dCSA₂₋₄ between semirigid and rigid fixation was observed. Between 4 and 6 weeks after surgery, only combined effects of fixation stability and gender variation influenced dCSA₄₋₆ ($p = 0.005$), whereas the gender alone ($p = 0.848$) and fixation stability alone ($p = 0.672$) variation did not.

At 6 weeks postoperatively, μ CT analysis of geometry, size, and mineralization of callus demonstrated a higher BV, CsAr, Jz, and BMD in male compared with female animals as well as in semirigid compared with rigid groups (Table 3). Additionally, microstructure differed between the groups (Fig. 6). Denser strut spacing and a higher Tb.N was observed for male compared with female animals and for semirigid compared with rigid groups (Table 4; Fig. 8).

Discussion

Clinically, gender and stability of fracture fixation have been reported to independently influence bone regeneration

Fig. 6A–D The figures show representative axial slices and three-dimensional reconstructions from μ CT scans of all groups at 6 weeks. (A) Group MR; (B) group FR; (C) group MS; and (D) group FS. The male and the semirigidly fixed animals show a larger extent of callus formation. Also the differences of the parameters Tb.N (more struts in

male and semirigid groups, especially in group MS) and Tb.Sp (larger spacing between struts in female and rigid groups) between the groups are visible in the axial slices. The white bar in each picture represents a scale of 1 mm.

Fig. 7 The figure shows intergroup comparison of bone volume (BV) between groups at 6 weeks followup. Whiskers represent single SD. p Values are from post hoc Bonferroni tests.

[2, 6, 10, 11, 16, 17], but it remains unclear whether these factors interact in a deleterious or synergistic way. Therefore, the purposes of the present pilot study were to generate research questions by comparing bone defect healing between middle-aged male and female rats under the influence of variable fixation stability using analysis from (1) mechanical properties of the callus at 6 weeks; (2) bony bridging of the defect; (3) in vivo callus development over time as well as (4) μ CT to determine callus mineralization, size, geometry, and microstructure.

Table 4. In vitro three-dimensional micro-CT microstructure metrics of the callus at 6 weeks

Factor	Groups	Tb.N (1/mm)	Tb.Sp (mm)	St.Th (mm)
Groups	MR	1.82 $\pm$ 0.70	0.78 $\pm$ 0.24	0.12 $\pm$ 0.02
	MS	3.40 $\pm$ 1.43	0.42 $\pm$ 0.20	0.15 $\pm$ 0.01
	FR	1.13 $\pm$ 0.35	1.17 $\pm$ 0.31	0.14 $\pm$ 0.03
	FS	1.38 $\pm$ 0.34	0.93 $\pm$ 0.16	0.14 $\pm$ 0.02
Gender	p*	p < 0.001	p < 0.001	p = 0.214
Fixation	p*	p = 0.007	p = 0.007	p = 0.625
Gender $\times$ fixation	p*	p = 0.042 [†]	p = 0.578	p = 0.227

All parameters are presented as mean $\pm$ single SD; *p values from two-way analysis of variance; [†] post hoc tests and intergroup comparison for Tb.N were performed (Fig. 8) because combined influence of gender and stability was significant regarding this parameter; Tb.N = callus strut number; Tb.Sp = callus strut spacing; Tb.Th = callus strut thickness.

Our study is limited by a number of factors. First, the number of animals was relatively small and we lost some data, further reducing the power of the study and increasing the risk of a Type II error. Second, except for the radiographic data, we examined a single time point in the healing process. Therefore, we can also draw no conclusions about the time course of bone defect healing and whether these differences would persist with time. Third, radiographic measurement of the CSA should be interpreted carefully because of the imprecision in detecting the

Fig. 8 The figure shows intergroup comparison of callus strut number (Tb.N) between groups at 6 weeks followup. Whiskers represent single SD. p values are from post hoc Bonferroni tests.

largest and smallest diameters in the two perpendicular radiographic views. We tried to minimize the resulting influence on group comparison using standardized views for every specimen and by using ex vivo μ CT at the end point. Fourth, the difference in weight of the animals between the groups (especially regarding gender) could not completely be avoided. This is important because with constant fixator stiffness, the weight difference leads to changes in mechanical load and interfragmentary movement and thereby of the healing outcome [3]. Therefore, to minimize the influences of weight, we chose an age of 12 months for the subject animals. At this age, the body weight of both genders is almost equal. Fifth, histologic analysis was not performed. Sixth, as a result of the nature of a small animal model, results must be interpreted carefully and cannot be transferred unrestrictedly to humans.

Semirigid fixation led to superior biomechanical properties compared with rigid fixation in both gender groups. This finding confirms similar results that were observed in our previous study in which bone healing in middle-aged individuals with varying fixator stabilities was compared with those of the young [24]. This could indicate a larger mechanical stimulus might be needed in middle-aged individuals to stimulate bone healing. Animal studies examining the remodeling of mechanically loaded intact bones give supporting evidence to this fact [18, 25]. It was also observed that stability had an influence on male groups to a larger extent than in females. The male groups had responded to the larger stimulus created in semirigid fixation by exhibiting a larger callus and better biomechanical properties earlier than the female groups. Whether this observation is somehow related to the findings of a previous study, in which a reduced mesenchymal stem cell quantity in the bone marrow of female animals was observed [22], will require further study.

In qualitative radiographic evaluation, bridging started earlier in the male compared with the female animals as reflected by the lower bridging score in females at 2 weeks. These observations may explain the higher TS and MTF in the male compared with the female animals. However, at the end of the experiments, when mechanical testing was performed, there was no difference in callus bridging between females and males. One could speculate that bone defect healing in female animals is simply delayed, especially in response to the mechanical input, leading to an immature callus tissue and inferior biomechanical properties. This is also well reflected in the callus microstructure in which female subjects had a less dense callus, indicated by the wider callus strut spacing, in comparison with male subjects.

Bridging results are well reflected by the quantitative radiographic evaluation over the course of healing in which callus formation in the males started earlier compared with the female animals. Furthermore, it was observed that the early (Weeks 0-2) callus CSA increase was larger in semirigid groups than in rigid groups, particularly in the male groups. It is known that callus size is a function of fixation instability [3, 6, 19], but somehow in the present study, this effect seems to be more prominent in the male animals. However, the underlying mechanism remains unclear and will require further study.

At the end point, the quantitative μ CT revealed strong differences in size (volume) and geometric distribution of mineralized callus tissue. Male individuals indicated superior callus properties by exhibiting a greater callus size, enhanced geometry, and denser microstructure. Additionally, semirigid groups had superior callus properties also indicated by changes in these outcome measures. From the analysis of cancellous bone, it is known that both Young's modulus and strength depend on bone volume fraction [4]. Using this as a guide for interpretation, with superior microstructural changes such as higher Tb.N and lower Tb.Sp, for a larger BV, and Jz observed in male individuals, we conclude the differences in biomechanical callus properties are purely the result of callus volume, geometry, and microstructure but not tissue mineralization. Mineralization is more likely influenced by age [13].

In conclusion, the present pilot study suggests that, independent of fixation stability, female gender apparently represents a risk factor for bone healing in middle-aged rats. Besides delayed callus formation in females, fixation instability's influence on callus properties seems to be larger in male animals. Our initial observations (Table 5) lead to the following questions: (1) Can delayed healing in female compared with male middle-aged rats be monitored by histology or in vivo μ CT? (2) Can it be observed in other species, too? (3) Are osteoblasts (or their precursors)

Table 5. Influences of gender and fixation stability on outcome parameters

Parameters		Gender	Fixation stability
Callus size	CsAr	Increased in males	Increased with semirigid fixation
	BV	Increased in males	Increased with semirigid fixation
Callus geometry	Jz	Increased in males	Increased with semirigid fixation
Callus microstructure	Tb.N	Increased in males	Increased with semirigid fixation
	Tb.Th	Not influenced	Not influenced
	Tb.Sp	Decreased in males	Decreased with semirigid fixation
Callus mineralization	TMD	Not influenced	Not influenced
Torsional strength	MTF	Increased in males	Increased with semirigid fixation
Torsional stiffness	TS	Increased in males	Increased with semirigid fixation
Energy to failure	E _{failure}	Not influenced	Increased with semirigid fixation

CsAr = callus cross-sectional area (6 weeks micro-CT); BV = callus volume; Jz = polar moment of inertia; Tb.N = callus strut number; Tb.Th = callus strut thickness; Tb.Sp = callus strut spacing; TMD = callus tissue mineral density; MTF = maximum torque at failure; TS = torsional stiffness; E_{failure} = energy to failure.

from female compared with male individuals less sensitive to mechanical stimulation? (4) Is cell count or cell attraction correlated to the delayed response and to the differences in callus size, microstructure, and geometry? Finally, we note any conclusions from our study would require corroboration in human studies.

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