Bone & Joint Research

Supplementary Material

10.1302/2046-3758.141.BJR-2024-0191.R2

Supplemental statistical analysis details

Data are reported as mean (SD) unless otherwise indicated.

External callus volume by quadrant

Callus volume was assessed in the 'around' zone only for differences based on only one within-subjects factor: 'quadrant'. There were no outliers in callus volume, as assessed by inspection boxplots. Shapiro-Wilk's test of normality showed that callus volume was normally distributed ($p \geq 0.083$) in all quadrants. Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(5) = 9.47$, $p = 0.096$. Callus volume was significantly different across the different time 'around' zones ($F(3,21) = 10.9$, $p < 0.001$, partial $\eta^2 = 0.609$). Bonferroni-adjusted pairwise comparisons were run to compare callus volume between all quadrants. Medial callus volume (1.12 ± 0.869 cm^3) and anterior callus volume (0.977 ± 0.694 cm^3) were not significantly different from each other ($p > 0.999$). Medial callus volume was significantly different from lateral callus volume (4.82 ± 3.16 cm^3) and posterior callus volume (3.98 ± 2.34 cm^3) (medial-lateral $p = 0.032$, medial-posterior $p = 0.047$). Anterior callus volume approached a significant difference from both lateral and posterior callus volume (anterior-lateral $p = 0.050$, anterior-posterior $p = 0.058$).

Callus BMD by zone and quadrant

Callus bone mineral density (BMD) was assessed for differences based on two within-subjects factors: 'zone' and 'quadrant'. There were no outliers in BMD, as assessed by examination of studentized residuals for values greater than ± 3. Shapiro-Wilk's test of normality on the studentized residuals showed that callus BMD was normally distributed ($p \geq 0.145$) in all zones and quadrants except for the 'anterior-around' segment ($p = 0.023$). Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(5) = 8.39$, $p = 0.141$. There was a statistically significant two-way interaction between 'zone' and 'quadrant', $F(3,21) = 14.8$, $p < 0.001$. Therefore, simple main effects were run.

Simple main effects of ‘zone’ were checked for each ‘quadrant’. In the ‘medial’ quadrant (near cortex), BMD was not statistically significantly different in the gap (‘in’; 614 ± 130 mgHA/cm^3) compared to ‘around’ (575 ± 74.9 mgHA/cm^3), $F(1,7) = 1.24$, $p = 0.302$. In the ‘lateral’ quadrant (far cortex), BMD was not statistically significantly different in the gap (IN; 510 ± 198 mgHA/cm^3) compared to ‘around’ (652 ± 99.9 mgHA/cm^3), $F(1,7) = 4.62$, $p = 0.069$. In the ‘anterior’ quadrant, BMD was not statistically significantly different for ‘in’ (470 ± 219 mgHA/cm^3) compared to ‘around’ (604 ± 63.3 mgHA/cm^3), $F(1,7) = 4.82$, $p = 0.064$. In the ‘posterior’ quadrant, BMD was statistically significantly different for ‘in’ (477 ± 177 mgHA/cm^3) compared to ‘around’ (701 ± 102 mgHA/cm^3), $F(1,7) = 21.6$, $p = 0.002$, a mean difference of 224 mgHA/cm^3 (95% CI 110 to 338).

Simple main effects of ‘quadrant’ were checked for each ‘zone’. For the interfragmentary gap zone (‘in’), BMD was statistically different based on quadrant, $F(3,21) = 5.01$, $p = 0.009$. Bonferroni-adjusted pairwise comparisons were only significant for BMD in the ‘in-medial’ segment versus the ‘in-posterior’ segment ($p = 0.025$). For the perifragmentary zone (‘around’), BMD was statistically different based on quadrant, $F(3,21) = 9.83$, $p < 0.001$. Bonferroni-adjusted pairwise comparisons were statistically significant for BMD in the following segment pairings: ‘around-medial’ versus ‘around-posterior’ ($p = 0.003$) and ‘around-anterior’ verses ‘around-posterior’ ($p = 0.032$).

Distortional strain by zone and quadrant

Distortional strain was assessed for differences based on two within-subjects factors: ‘zone’ and ‘quadrant’. There were no outliers, as assessed by examination of studentized residuals for values greater than ± 3. Shapiro-Wilk's test of normality on the studentized residuals showed that distortional strain was normally distributed in all eight zone/quadrant locations (all $p \geq 0.067$). Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(5) = 2.46$, $p = 0.785$. There was not a statistically significant two-way interaction between ‘zone’ and ‘quadrant’, $F(3,21) = 2.10$, $p = 0.131$. The main effect of ‘zone’ showed that there was a statistically significant difference in distortional strain between the IN and AROUND locations, $F(1,7) = 9.77$, $p = 0.017$. The main effect of ‘quadrant’ showed a statistically significant difference in distortional strain across the four locations, $F(3,21) = 43.7$, $p < 0.001$. Bonferroni-adjusted pairwise comparisons were significant ($p \leq 0.013$) for all quadrant pairings except for between ‘anterior’ and ‘posterior’ ($p = 1.000$).

Volumetric strain by zone and quadrant

Volumetric strain was assessed for differences based on two within-subjects factors: ‘zone’ and ‘quadrant’. Peak volumetric strains were compressive (negative values due to fracture gap closure) so the absolute value was evaluated for convenience in plotting and analysis. There were no outliers, as assessed by examination of studentized residuals for values greater than ± 3. Shapiro-Wilk's test of normality on the studentized residuals showed that volumetric strain was normally distributed ($p \geq 0.422$) in three of the eight zones and quadrants. Volumetric strains deviated from normality for the ‘in-medial’ ($p = 0.002$), ‘around-medial’ ($p < 0.001$), ‘in-lateral’ ($p = 0.030$), ‘in-anterior’ ($p = 0.015$), and ‘around-anterior’ ($p = 0.029$) locations. Analysis of variance (ANOVA) is considered robust

to deviations from normality when the distribution skew is consistent, as was the case in this data (positive skew), so the results of the two-way ANOVA were evaluated without transformation. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(5) = 4.73$, $p = 0.455$. There was a statistically significant two-way interaction between 'zone' and 'quadrant', $F(3,21) = 8.98$, $p < 0.001$. Therefore, simple main effects were run.

Simple main effects of 'zone' were checked for each 'quadrant'. In the 'medial' quadrant (near cortex), volumetric strain was not statistically significantly different in the gap ('in'; 13% ± 5.6%) compared to 'around' (14% ± 12%), $F(1,7) = 0.561$, $p = 0.478$. In the 'lateral' quadrant (far cortex), volumetric strain was significantly different in the gap ('in'; 28% ± 6.5%) compared to 'around' (16% ± 5.5%), $F(1,7) = 16.9$, $p = 0.005$, a mean difference of 12% (95% CI 1.9% to 18%). In the 'anterior' quadrant, volumetric strain was statistically significantly different for 'in' (9.6% ± 4.8%) compared to 'around' (9.6% ± 3.5%), $F(1,7) = 38.6$, $p < 0.001$, a mean difference of 11% (95% CI 6.6 to 15%). In the 'posterior' quadrant, volumetric strain was significantly different for 'in' (17% ± 1.4%) compared to 'around' (11% ± 1.4%), $F(1,7) = 180$, $p < 0.001$, a mean difference of 6.7% (95% CI 5.5% to 7.9%).

Simple main effects of 'quadrant' were checked for each 'zone'. For the interfragmentary gap zone ('in'), volumetric strain was statistically different based on quadrant, $F(3,21) = 12.7$, $p < 0.001$. Bonferroni-adjusted pairwise comparisons were significantly different between the 'in-medial' and 'in-lateral' ($p = 0.022$) and between 'in-lateral' and 'in-posterior' ($p = 0.087$); all other pairwise comparisons were non-significant ($p \geq 0.203$). For the perifragmentary zone ('around'), volumetric strain did not significantly depend on quadrant, $F(3,21) = 1.510$, $p = 0.241$, so no pairwise comparisons were performed.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	N/A - no group comparisons Methods
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Methods N/A no group comparisons
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Methods N/A Methods/Results
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	No groups, no controls N/A
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	N/A
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Methods Methods
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods Methods -- Supplemental Digital Content
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods N/A
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Methods Methods Methods Methods
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Results N/A

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction Data reuse consistent with 3Rs -- see Methods
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Methods
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Methods N/A N/A
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion Discussion
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Introduction
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Methods
Data access	20	Provide a statement describing if and where study data are available.	Data sharing
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	ICMJE COI statement Funding statement