Bone & Joint Research

Supplementary Material

10.1302/2046-3758.114.BJR-2020-0308.R2

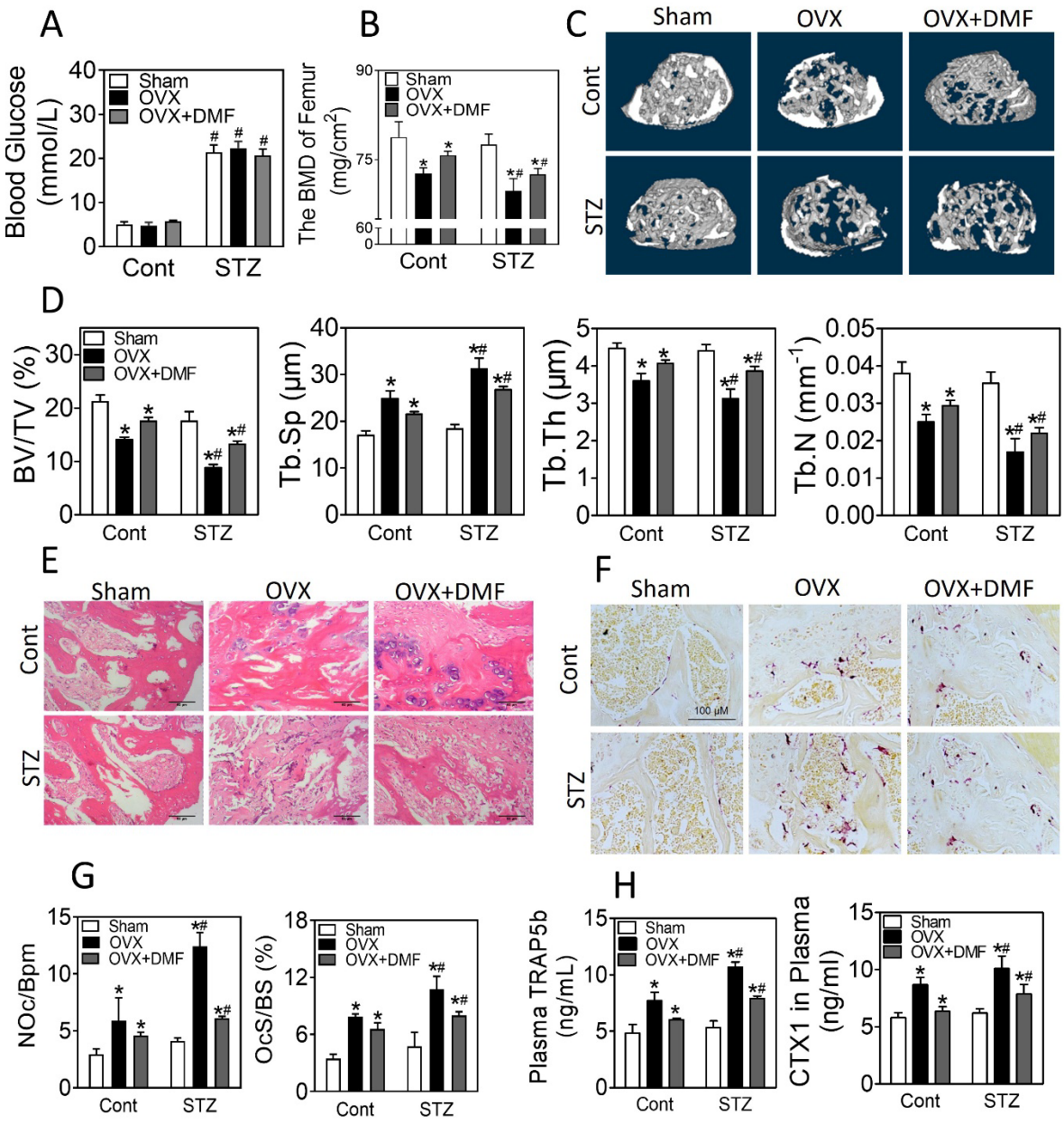

Fig a. N,N-dimethylformamide (DMF) attenuates high glucose-induced susceptibility to osteoporosis in vivo. C57BL/6 mice were treated with streptozotocin and subjected to ovariectomy (OVX) to establish the glucose-related osteoporosis mouse model. The mice were then treated with DMF (30 mg/kg) to evaluate its function in osteoporosis. a) The blood glucose level in the mice. b) Dual-energy X-ray absorptiometry analysis of femora of mice. c) Micro-CT analysis in the mice. d) Trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone volume per tissue volume (BV/TV). Representative histological images (200×) of e) haematoxylin and

eosin (H&E) staining and f) tartrate-resistant acid phosphatase (TRAP) staining. g) Osteoclast number (NOc) and osteoclast surface (OcS) in femur sections with TRAP staining normalized to the bone perimeter (Bpm) and bone surface (BS), respectively. h) The plasma levels of bone metabolism biomarkers, including TRAP5b and c-terminal telopeptides of type 1 (CTX1) measured by enzymelinked immunoabsorbent assay (ELISA) in the mice (n = 5). Data were presented as mean and standard deviation. Statistically significant differences were indicated (two-way analysis of variance). *p < 0.05 versus sham of the same streptozotocin treatment, #p < 0.05 versus control with the same OVX and/or DMF treatment.

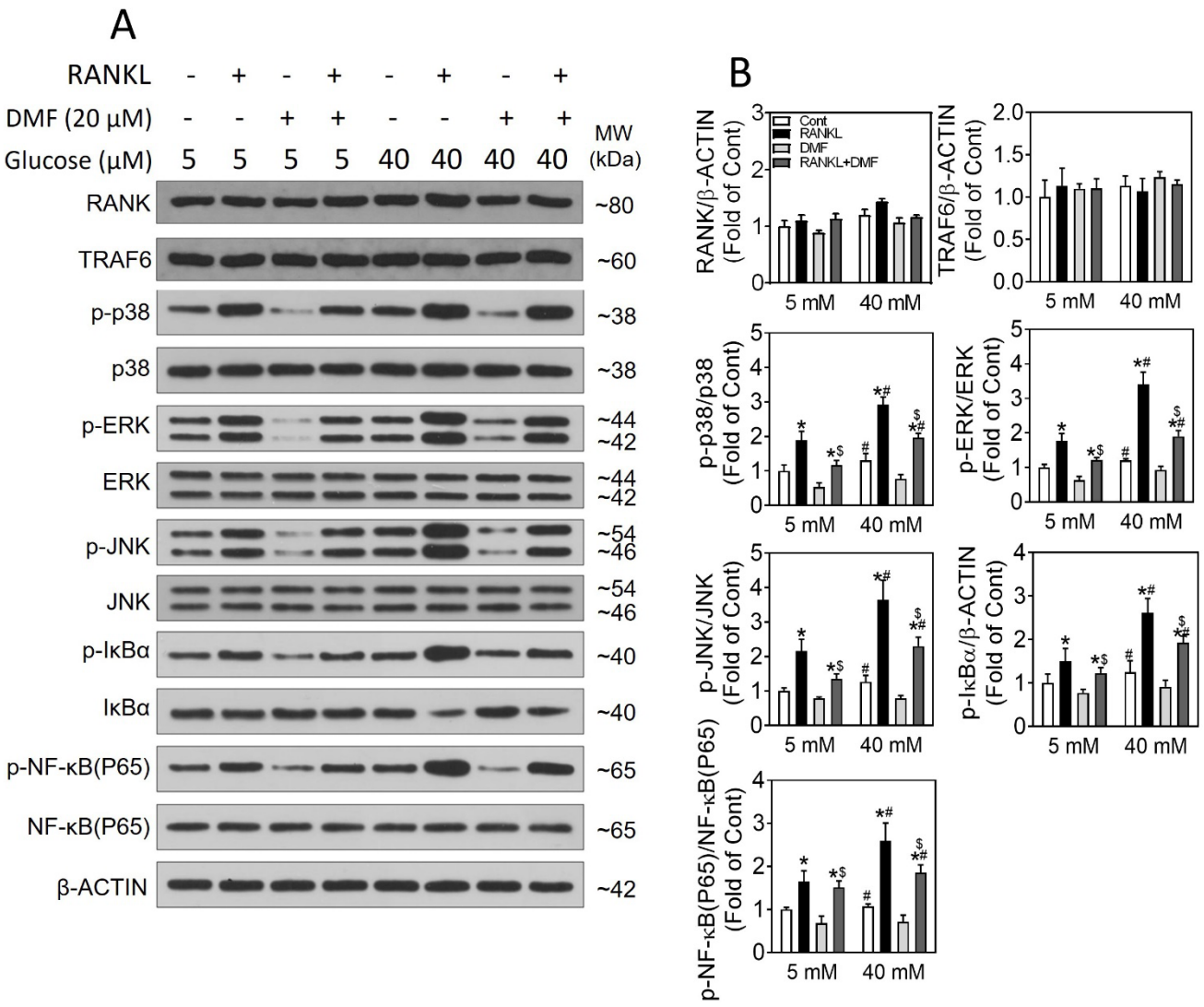

Fig b. N,N-Dimethylformamide (DMF) inhibits high glucose-activated mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signalling. The glucose-treated RAW 264.7 cells were treated with receptor activator nuclear factor κB ligand (RANKL), DMF, or their combination for 30 minutes. a) The expression levels of RANKL, TNF receptor associated factor 6 (TRAF6), P38, ERK, c-Jun N-terminal kinase (JNK), NF-κB (p65), IκBα, β-Actin, and phosphorylated P38, extracellular signal-related kinase (ERK), JNK, NF-κB (p65), and IκBα were measured by Western blot. b) Western blot results were quantified by ImageJ software (National Institutes of Health, USA). Data were presented as mean and standard deviation of three independent experiments. *p < 0.05 versus control of the same glucose concentration, #p < 0.05 versus 5 mM glucose with the same DMF and/or RANKL treatment, $p < 0.05 versus the same glucose concentration plus RANKL treatment (two-way analysis of variance).

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).	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
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
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.	Methods
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).	Methods
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
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
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
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
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

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
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.	Ethical review statement
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
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
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).	Methods
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