

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Title
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Abstract
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.	Line 50-72
		b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Line 62-72
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Line 57-72
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Line 90-92
Study design	6	For each experiment, give brief details of the study design including: <ul style="list-style-type: none"> a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	<ul style="list-style-type: none"> a. n=5-6 in each experimental group (Line 109-121), n=7-9 in each experimental group (Line 125-129), n=12-13 in each experimental group (Line 132-138), and n=4-5 in each experimental group (Line 148-154). b. N/A c. Line 86-88

Experimental procedures	7	<p>For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:</p> <ul style="list-style-type: none"> a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used). 	Line 109-121, Line 125-129, Line 132-138, and Line 142-147
Experimental animals	8	<ul style="list-style-type: none"> a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. 	Line 83-86

The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010¹

Housing and husbandry	9	<p>Provide details of:</p> <p>a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).</p> <p>b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).</p> <p>c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.</p>	Line 83-92
Sample size	10	<p>a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.</p> <p>b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.</p> <p>c. Indicate the number of independent replications of each experiment, if relevant.</p>	<p>a. Total number = 36 and n=6 in each experimental group (Line 109-121). Total number = 24 and n=6 in each experimental group (Line 125-129). Total number = 26 and n=8-9 in each experimental group (Line 119-123). Total number = 26 and n=13 in each experimental group (Line 132-138). Total number = 9 and n=4-5 in each experimental group (Line 148-154).</p> <p>b. Line 182-188</p> <p>c. Repeat 2 times to achieve the number of rats (n=8-9) in experiment (Line 125-129).</p>
Allocating animals to experimental groups	11	<p>a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.</p> <p>b. Describe the order in which the animals in the different experimental groups were treated and assessed.</p>	<p>a. Rats were divided into several groups to have comparable body weight the day prior to oral administration.</p> <p>b. The order was randomized.</p>

Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Line 100-105, Line 132-138, and line 155-179
Statistical methods	13	<ul style="list-style-type: none"> a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach. 	Line 189-194 and Fig. 1-5
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	Fig1 and Fig2 (Basal GLP-1 levels)
Numbers analysed	15	<ul style="list-style-type: none"> a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%²). b. If any animals or data were not included in the analysis, explain why. 	<ul style="list-style-type: none"> a. Fig. 1-5 b. In some experiments, we have to omit postprandial response data because some of rats did not allow oral gavage during experiments.
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Fig. 1-5
Adverse events	17	<ul style="list-style-type: none"> a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events. 	<ul style="list-style-type: none"> a. No important adverse events b. We collected blood from tail vein to avoid making rat injured too much.
DISCUSSION			
Interpretation/scientific implications	18	<ul style="list-style-type: none"> 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 any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results². c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research. 	<ul style="list-style-type: none"> a. Throughout b. Line 378-380 c. Although the current research cannot be replaced by non-animal study because “in vivo GLP-1 response” are not reproduced in any other models, we

			reduced animal numbers by repeated collection of blood sample from identical rats.
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Line 395-397
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	Acknowledgements

References:

1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.

