



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).	a. Comparison groups: Standard diet (SD) group, high-fat diet (HFD) group, HFD + low-dose sodium butyrate (HFD+LNaB, 200 mg/kg), HFD + high-dose sodium butyrate (HFD+HNaB, 400 mg/kg). SD served as blank control and HFD as model control. (In Materials and Methods Section 2.3.)
		b. Individual mice were allocated to the experimental groups (n = 6 mice/group). (In Materials and Methods Section 2.3.)
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 <i>a priori</i> sample size calculation, if done.	a. Twenty-four male C57BL/6 mice were used, with 6 mice allocated to each of the four groups. (In Materials and Methods Section 2.3.)
		b. No <i>a priori</i> sample size calculation was performed. Group size of 6 was selected by referencing standard sample settings of analogous MASLD intervention animal studies, balancing sufficient biological replicates and animal welfare constraints. (In Materials and Methods Section 2.11.)
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 <i>a priori</i> . 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.	a. Specific inclusion/exclusion criteria were not explicitly reported in the current manuscript.
		b. No animals, experimental units or data points were excluded from any group during analysis; no mortality, sampling failure or invalid detection samples occurred. c. The <i>in vivo</i> sample size was n = 6 mice per group. (In Materials and Methods Sections 2.3.)

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.	a. Random allocation was applied to divide 24 mice into four groups after acclimatisation; random number table method was used to generate randomisation sequences for group assignment. (In Materials and Methods Section 2.3.)
			b. Confounder minimisation strategies: mice were evenly distributed into cages with unified housing density; cages were randomly placed on rack layers to eliminate bias from light and temperature gradients; blood collection, OGTT, IPITT and tissue harvest were conducted in randomised operation order to avoid temporal confounding factors. All major confounders were controlled. (In Materials and Methods Section 2.3.)
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).	1. Group allocation stage: researchers were aware of group assignments.2. Animal housing and oral intervention stage: animal caretakers were aware of group assignments.3. Phenotypic measurement (body weight monitoring, OGTT, IPITT): operators were aware of group assignments.4. Histological staining, biochemical detection, western blot, RT-qPCR detection: sample IDs were anonymised to mask group information, and laboratory technicians performed tests under blinded conditions.5. Data statistics and image quantitative analysis: analysts were blinded to group allocation. (In Materials and Methods Section 2.3 – 2.6 and 2.8.)
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.	Outcomes included body weight, serum and hepatic lipid indices, glucose tolerance and insulin sensitivity, hepatic glycogen and histology, and inflammatory markers. (In Materials and Methods Sections 2.4 – 2.6 and 2.10; Results Sections 3.6 – 3.9.) b. Primary outcome measure for hypothesis testing: hepatic triglyceride (hepatic TG), which was used to characterise core MASLD pathological phenotype and support experimental endpoint evaluation. (In Materials and Methods Section 2.6.)

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.	a. Statistical methods for each analysis: unpaired two-tailed Student's t-test for two-group comparisons; one-way ANOVA followed by appropriate post-hoc tests for multiple-group comparisons. GraphPad Prism was used for data plotting and statistical analysis; microplate reader supporting software was applied for biochemical absorbance quantification. (In Materials and Methods Section 2.11.) b. Formal tests for normality and homogeneity of variance were not separately described. Non-parametric post-hoc corrections were adopted if datasets violated parametric test assumptions. Statistical significance threshold was set as two-sided $p < 0.05$ for all analyses. (In Materials and Methods Section 2.11.)
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.	a. Species-specific animal details: Species: mouse; strain: C57BL/6; no substrain specified; sex: male; age: 4 weeks old at the start of experiment; initial body weight was maintained within a consistent range. (In Materials and Methods Section 2.3.) b. Animal provenance: Hangzhou Ziyuan Laboratory Animal Science and Technology Co., Ltd. Health status: SPF-grade pathogen-free mice; no genetic modification; no prior surgical operations or drug administration before experimental initiation. (In Materials and Methods Section 2.3.)
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).	a. Mice received SD or HFD (D12492); NaB was administered by daily intragastric gavage at 200 or 400 mg/kg, followed by metabolic testing, euthanasia, and tissue collection. (In Materials and Methods Sections 2.3 – 2.6 and 2.10.) b. HFD feeding lasted 24 weeks, with daily NaB treatment from weeks 16 – 24; model assessment was performed at week 16 and endpoint sampling at week 24. (In Materials and Methods Sections 2.3 – 2.4.) c. Housing environment: Controlled temperature $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, 12-hour light-dark cycle, ad libitum

			access to food and water; all mice underwent a 1-week acclimatisation period without any intervention before formal experiments. (In Materials and Methods Section 2.3.)
			d. Rationale for procedures: Long-term HFD feeding induces MASLD and hepatic insulin resistance; oral NaB administration mimics physiological exposure to gut microbiota-derived short-chain fatty acid in vivo, to investigate its therapeutic effects and underlying molecular mechanisms against hepatic steatosis and insulin resistance. (In Materials and Methods Section 2.3 – 2.4.)
Results	10	For each experiment conducted, including independent replications, report: - Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and standard deviation, or median and range). - If applicable, the effect size with a confidence interval.	Data are reported as mean $\pm$ standard deviation, with n values and statistical comparisons provided in the corresponding figures/tables. (In Materials and Methods Section 2.11 and Results Sections 3.6 – 3.9.) b. Not applicable.(The experiment did not involve statistical analysis of confidence intervals.)

Note:1.This manuscript contains both in vitro cellular experiments and in vivo animal studies. The ARRIVE guidelines only apply to live animal in vivo assays; cell culture data are not required for this checklist.2.All animal procedures were approved by the institutional animal ethics committee (Approval No. P-SL-2025-024), complying with national and international guidelines for laboratory animal care and use.3.No unexpected animal mortality, sample loss or data exclusion occurred during the whole experiment; random group allocation and blinded sample detection were implemented to minimise experimental bias.