

The ARRIVE guidelines - Examples Animal Research: Reporting In Vivo Experiments

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The ARRIVE guidelines are designed to improve the reporting of animal research. This document demonstrates how the ARRIVE guidelines can be used in practice to report animal research, by providing specific examples for each point of the guidelines. The examples given are from a wide range of research using a range of animal species. Each example has been chosen for meeting the criteria of the checkpoint mentioned and does not imply the entire manuscript complies with the ARRIVE guidelines.

ITEM	RECOMMENDATION	EXAMPLE
Title	1 Provide as accurate and concise a description of the content of the article as possible.	Thoracic cage plasticity in prepubertal New Zealand white rabbits submitted to T1-T12 dorsal arthrodesis: computed tomography evaluation, echocardiographic assessment and cario-pulmonary measurements. (Canavese et al., 2013).
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.	<p>BACKGROUND AND PURPOSE: Asthma is an inflammatory disease that involves airway hyperresponsiveness and remodelling. Flavonoids have been associated to anti-inflammatory and antioxidant activities and may represent a potential therapeutic treatment of asthma. Our aim was to evaluate the effects of the sakuranetin treatment in several aspects of experimental asthma model in mice.</p> <p>EXPERIMENTAL APPROACH: Male BALB/c mice received ovalbumin (i.p.) on days 0 and 14, and were challenged with aerolized ovalbumin 1% on days 24, 26 and 28. Ovalbumin-sensitized animals received vehicle (saline and dimethyl sulfoxide, DMSO), sakuranetin (20 mg kg⁻¹per mice) or dexamethasone (5 mg kg⁻¹ per mice) daily beginning from 24th to 29th day. Control group received saline inhalation and nasal drop vehicle. On day 29, we determined the airway hyperresponsiveness, inflammation and remodelling as well as specific IgE antibody. RANTES, IL-5, IL-4, Eotaxin, IL-10, TNF-α, IFN-γ and GMC-SF content in lung homogenate was performed by Bioplex assay, and 8-isoprostane and NF-κB activations were visualized in inflammatory cells by immunohistochemistry.</p>

	<p>KEY RESULTS: We have demonstrated that sakuranetin treatment attenuated airway hyperresponsiveness, inflammation and remodelling; and these effects could be attributed to Th2 pro-inflammatory cytokines and oxidative stress reduction as well as control of NF-κB activation.</p> <p>CONCLUSIONS AND IMPLICATIONS: These results highlighted the importance of counteracting oxidative stress by flavonoids in this asthma model and suggest sakuranetin as a potential candidate for studies of treatment of asthma. (Toledo et al., 2013)</p>
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INTRODUCTION

Background	3	<p>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.</p>	<p>The increasing prevalence of type 2 diabetes is cause for concern, and has spurred efforts to identify novel peptides with valuable properties for diabetes treatment [1]. Type 2 diabetes is characterized by both resistance of target tissues to the actions of insulin and impaired β-cell function [2], [3]. Studies in genetically modified mice have suggested that defects in insulin/IGF signaling in the β-cell contribute to β-cell failure [4], thereby establishing a causal link between insulin resistance and impaired β-cell function. One attractive scenario is that insulin and IGFs exert their effects through a common effector, acting on DNA transcription in β-cells [5]. Forkhead box (Fox)-containing transcription factors of the O sub-class (FoxO) are prominent transcriptional effectors of insulin and IGF signaling in β-cells [6]. FoxO1 inhibits β-cell proliferation in insulin-resistant states [7] as well as in response to growth factors [8], protects β-cells against hyperglycemia-induced oxidative stress [9], and controls energy metabolism in β-cells [10]. In view of the role of FoxO1 in β-cell compensation to insulin resistance [11], we reasoned that investigation of FoxO1 target genes could reveal mechanisms underlying β-cell failure in the context of insulin resistance. To this end, we carried out gene profiling analyses in INS832/13 cells [10]. Our genomic analysis led to the identification of nephroblastoma overexpressed gene (Nov, also known as Ccn3) as a novel FoxO1 target. The role of Ccn3 in β-cells has never been explored. (Paradis et al., 2013)</p>
		<p>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.</p>	<p>For this purpose, we selected a pilocarpine model of epilepsy that is characterized by robust, frequent spontaneous seizures acquired after a brain insult [15, 16, 17, 18] well-described behavioral abnormalities [18] and poor responses to antiepileptic drugs [19]. These animals recapitulate several key features of human temporal lobe epilepsy, the most common type of epilepsy in adults [1, 2]. (Hunt et al., 2013)</p>
Objectives	4	<p>Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.</p>	<p>Therefore, the objectives of this study were to determine whether URB597, a selective inhibitor of FAAH, increases retinal ganglion cell (RGC) survival in an axotomy model of optic nerve injury, and to determine the contribution of CB1 and CB2 to the survival-promoting effects of URB597 in the retina. (Slusar et al., 2013)</p>

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.

All animal experiments conformed to the British Home Office Regulations (Animal Scientific Procedures Act 1986; Project License PPL70/7162 to Prof Andrew Rice) and International Association for the Study of Pain guidelines [90] for the care and use of animals. (Huang et al., 2013)

Study design 6 For each experiment, give brief details of the study design including:

Three groups of 20 mice each were studied: A. Wild type fed AIN 93 G diet; B. Wild type fed AIN 93 G diet supplemented with 0.1% quinine HCl; C. Wild type fed AIN 93 G diet supplemented with 0.01% quinine HCl. (Cettour-Rose et al., 2013)

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).

In experiments for mechanical hypersensitivity development and pharmacological studies, animals were randomized into treatment groups by picking numbers out of a hat. In experiments for thigmotaxis and burrowing, random cage assignment to treatments or TNT/sham surgeries were applied by picking numbers out of a hat. (Huang et al., 2013)

c. The experimental unit (e.g. a single animal, group or cage of animals).

In the study, n refers to number of animals, with five acquisitions from each slice, with a maximum of three slices obtained from each experimental animal used for each protocol (six animals each group). (Grasselli et al., 2013)

d. A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.

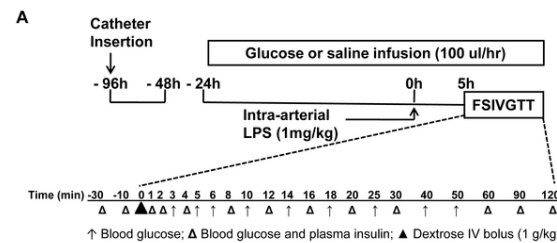


Figure 1. Experimental timelines. (A) Chronically catheterized mice (femoral artery and vein) were given three days to recover from surgery and infused intravenously (iv) with either saline or glucose for 24 hours prior to intra-arterial administration of either lipopolysaccharide (LPS; 1 mg/kg) or vehicle. Five hours after LPS or vehicle administration a frequently sampled intravenous glucose tolerance test (FSIVGTT) was performed over a two hour period. An iv glucose bolus of 1 g/kg D50 was given over approximately 15 sec (▲). Subsequently, multiple samples of either blood glucose and plasma insulin (Δ) or glucose alone (↑) were taken from the arterial catheter at the times identified. (Watanabe et al., 2012)

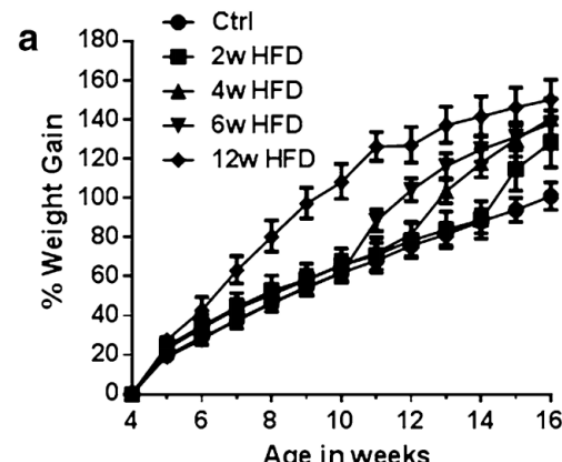
Experimental procedures	<p>7 For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:</p> <p>a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).</p> <p>b. When (e.g. time of day).</p> <p>c. Where (e.g. home cage, laboratory, water maze).</p> <p>d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).</p>	<p>All rats were chronically implanted with epidural silverball electrodes under inhalation anaesthesia (isoflurane 2–3% mixed with 30% oxygen (O₂) and 70% nitrous oxide (N₂O)). Prior to surgery, rats were given an i.p. injection of 5 mg/kg Carprophen (Rimadyl) as analgetic. (Jung et al., 2013)</p> <p>Except the burrowing assay, which was conducted from the beginning of the dark cycle, all other behavioural experiments were conducted in the light phase. (Huang et al., 2013)</p> <p>The animals were tested in a square shaped apparatus which comprised of an E-shaped object area, which could be adapted for different contexts, abutting an E-shaped holding area, which was stable (Fig. 1). The apparatus was 59 cm long and 59 cm wide. Opaque guillotine doors divided the two areas (outer arm doors: 12 cm; central arm door: 24 cm) which could be opened and closed by the experimenter. (Ameen-Ali et al., 2012)</p> <p>The subcutaneous (s.c.) route may be used for agents to prolong duration of action, the intravenous route (i.v.) avoids issues of first pass metabolism (number of doses restricted due to potential damage to veins), the intraperitoneal (i.p.) route using 27–30g needles when repeated injections are required, or by oral (p.o.) gavage in volumes of less than 5ml/kg. (Al-Izki et al., 2012)</p>
Experimental animals	<p>8 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).</p> <p>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.</p>	<p>Male C57BL/6J mice (25.3 ± 1.4 g), aged 8–12 weeks, were included (n = 40). (Van Dijk et al., 2013)</p> <p>Twenty-two male Sprague–Dawley rats (<i>Rattus norvegicus</i>; Harlan Laboratories, Indianapolis, IN, USA) were obtained and acclimatized for at least 48 h. Vendor health reports indicated that the rats were free of known viral, bacterial and parasitic pathogens. (Katayama et al., 2013)</p>

Housing and husbandry	<p>9 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>	<p>Animals were housed with an inverse 12 hours day-night cycle with lights on at 8:30pm in a temperature (22±1°C) and humidity (55±5%) controlled room. Prior to surgery the animals were housed pairwise in type 4 cages filled with Lignocel® (hygiene animal bedding) enriched with nest boxes and horizontal tubes for climbing. (Jung et al., 2013)</p> <p>All mice were allowed free access to water and a maintenance diet containing 0.75% calcium (EURodentDiet 22%; PMI Nutrition International, LLC, Brentwood, MO, USA) in a 12-hour light/dark cycle, with room temperature at 21±2 °C. All cages contained wood shavings, bedding and a cardboard tube for environmental enrichment. (Meakin et al., 2013)</p> <p>During the postoperative period, pain was relieved by a subcutaneous administration of carprofen (Rimadyl, Pfizer Animal Health, West Dundee, Great Britain; 5 mg/kg twice daily for 5 days). An intramuscular injection of enrofloxacin (Baytril_ 5 %, Bayer Animal Health, Kiel, Germany; 5 mg/kg twice daily) was administered for the prevention of infection during the week following surgery. (Canavese et al., 2013)</p>
Sample size	<p>10 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>Twenty eight healthy rats were divided into four groups of seven each. Animals of group I received distilled water (0.1 ml/day) and served as control, whereas group II animals received only ISO at 100 mg/kg. Animals of group III and IV were treated with test alkaloid at pre-standardized dose of 40 mg/kg (p.o.) daily for 7 days. (Panda et al., 2013)</p> <p>Sample size calculations were performed in STATA/IC 10 (StataCorp, College Station, Texas, USA) with the sampsi function. Stratified meta-analysis of hypothermia treatment in SHR (10) reported a normalized mean effect size of 49% [standard deviation (SD) = 28%]. To reject the null hypothesis that pethidine does not attenuate this effect, we predicted a normalized mean effect size of hypothermia in the presence of pethidine of 29%. To achieve power = 0.8 and alpha = 0.05 to detect this difference would require a total of 60 animals. (Sena et al., 2013)</p> <p>The experiment was repeated, and data were pooled. (Grasselli et al., 2013)</p>
Allocating animals to experimental groups	<p>11 a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.</p>	<p>For experiments using animals without surgery, rats were ranked in ascending order according to pre-test levels of burrowing and allocated to treatment groups in order i.e. rat 1 group 1, rat 2 group 2, rat 3 group 3, rat 4 group 1, etc., thus ensuring the median of each group was similar prior to testing. (Andrews et al., 2011)</p>

	b. Describe the order in which the animals in the different experimental groups were treated and assessed.	For the thigmotaxis experiment, sequences of A–B–C then C–B–A (letters assigned to mask the cage labels during testing) were used to select animals. (Huang et al., 2013)
Experimental outcomes	12 Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Two primary outcome measures were analyzed: overall performance on the MWM (days 12–16) and the numbers of surviving CA2-3 cells. In addition, three secondary outcome measures were evaluated: terminal performance in the MWM (days 15–16), rate of learning the MWM (slope of days 12–14), and MWM probe trial. (Wang et al., 2013)
Statistical methods	13 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.	Kaplan–Meier survival analysis was performed and student t-test for normally distributed data (i.e., body weight, ABGA results, blood pressure, heart rate, body temperature, asphyxia time, CPR duration, and lactate) or Mann–Whitney U tests for non-normally distributed data (i.e., western blot results and immunohistochemistry results) were performed to compare the differences of baseline characteristics, and expressions of cleaved caspase-3 and acetylated histone H3. For NDS, repeated measures analysis of variance test and Bonferroni posthoc test was performed. (Hyuk et al., 2013) For each test, the experimental unit was an individual animal. (Podrini et al., 2013) Test for normality was performed by Kolmogorov–Smirnov test. (Hyuk et al., 2013)

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).	The animals' health status was monitored throughout the experiments by a health surveillance programme according to Federation of European Laboratory Animal Science Associations (FELASA) guidelines. The mice were free of all viral, bacterial, and parasitic pathogens listed in the FELASA recommendations, except for <i>Helicobacter</i> species. (Jirkof et al., 2013)
Numbers analysed	15 a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50% ²).	Sixteen MPV-positive samples were from the group of young adults, with a positive rate of 13.9% (16/115), and the other four positive samples were detected in the middle-aged adult group, with a positive rate of 11.4% (4/35) (Table 1). (Wang et al., 2013)

	<p>b. If any animals or data were not included in the analysis, explain why.</p> <p>Thirty-eight rats were utilized for this study and 30 were included and completed. Eight animals were excluded, including 5 rats which were not resuscitated (persistent VF during ECLS) and another 3 rats were excluded because of instrumentation or technical failure during animal preparation. (Rungtatscher et al., 2013)</p>																																																
<p>Outcomes and estimation</p> <p>16 Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).</p>	<p>In accordance with the ARRIVE guidelines (Kilkenny et al. 2010), we have reported measures of precision, confidence, and n to provide an indication of significance.</p> <p>Cages of mice were randomly assigned to each of the five groups (n = 30 per group).</p> <p>Fig. 2 Comparison of body weight of C57BL/6NTac male mice following varying durations of exposure to HFD. a Percentage body weight gain from 4 to 16 weeks of age relative to the starting body weight at 4 weeks of age which ranged from 12.2 to 20.3 g (mean = 16.9 g). Data are presented as mean percentage weight gain \pm SEM. (Podrini et al., 2013)</p>  <table border="1"> <caption>Estimated data for Figure 2a: % Weight Gain vs Age in weeks</caption> <thead> <tr> <th>Age (weeks)</th> <th>Ctrl</th> <th>2w HFD</th> <th>4w HFD</th> <th>6w HFD</th> <th>12w HFD</th> </tr> </thead> <tbody> <tr><td>4</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr> <tr><td>6</td><td>25</td><td>30</td><td>35</td><td>40</td><td>45</td></tr> <tr><td>8</td><td>45</td><td>50</td><td>55</td><td>60</td><td>65</td></tr> <tr><td>10</td><td>65</td><td>70</td><td>75</td><td>80</td><td>85</td></tr> <tr><td>12</td><td>85</td><td>90</td><td>95</td><td>100</td><td>105</td></tr> <tr><td>14</td><td>105</td><td>110</td><td>115</td><td>120</td><td>125</td></tr> <tr><td>16</td><td>125</td><td>130</td><td>135</td><td>140</td><td>145</td></tr> </tbody> </table>	Age (weeks)	Ctrl	2w HFD	4w HFD	6w HFD	12w HFD	4	0	0	0	0	0	6	25	30	35	40	45	8	45	50	55	60	65	10	65	70	75	80	85	12	85	90	95	100	105	14	105	110	115	120	125	16	125	130	135	140	145
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<p>Adverse events</p> <p>17 a. Give details of all important adverse events in each experimental group.</p> <p>b. Describe any modifications to the experimental protocols made to reduce adverse events.</p>	<p>In four surviving animals, lower extremity ulcers developed but were effectively treated with local standard triple antibiotic ointment (bacitracin, neomycin, and Polymyxin B) and cohesive bandages. (Van Gorp et al., 2013)</p> <p>As mortality with multiple surgeries was significantly higher in aged animals than young animals, aged animals only underwent a single surgery in which RGCs were labelled either from the SC (aged control non-axotomized animals) or from the optic nerve stump at the time of axotomy. (Slusar et al., 2013)</p>																																																

DISCUSSION

Interpretation/ scientific implications	18 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 results ² . 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.	The aim of our study was to verify if the pharmacological manipulation of the endocannabinoid system could be effective in the modulation of abnormal eating behaviour developed by female rats in a confirmed rat model of BED, in which binge eating behaviour is induced in animals by giving them a sporadic (3 days week-1) and limited (2 h) access to a high-fat diet (margarine) in addition to a continuous access to chow and water (HR group). In these animals, the intake of margarine becomes significantly greater than those of animals with limited daily access to margarine (LR group), and remains stable over prolonged periods of time (Corwin and Buda-Levin, 2004; Corwin and Wojnicki, 2006). In our study, the effect of rimonabant on the bingeing group could be related to its capacity to block dopamine release in the nucleus accumbens shell that might be induced by the consumption of margarine, and by a possible enhancement in the tone of the endocannabinoid system. Chronic exposure to high-fat palatable diet was found to decrease the expression of CB1 receptors in the nucleus accumbens (Harrold et al., 2002). Accordingly, Bello et al. (2012) reported a reduction in CB1 receptor density in the same central area in an animal model of BED. (Scherma et al., 2013) A limitation of this study is the fact that the estrous cycle stage of the female rats was not determined, since the stage of the estrus cycle at the time of tissue collection could potentially have affected gene expression levels. (Ong et al., 2013) The new apparatus shows potential for considerably reducing the number of animals used in memory tasks designed to detect potential amnesic properties of new drugs approximately 43,000 animals have been used in these tasks in the past 5 years but with the application of the continual trials apparatus we estimate that this could have been reduce to 26,000. (Ameen-Ali et al., 2013)
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.	Establishing anesthesia-independent settings for probing rodent analogues to the human MMN are important for facilitating the detection of therapeutic targets at the cellular level. Knowledge of these targets is likely to help guiding the development of drugs for treating the disorders that have been shown to be accompanied with reduced MMN responses, such as schizophrenia. (Jung et al., 2013)
Funding	20 List all funding sources (including grant number) and the role of the funder(s) in the study.	The research leading to these results is part of the European Collaboration, which has received support from the Innovative Medicines Initiative Joint Undertaking, under Grant agreement 115007, resources of which are composed of financial contribution from the European Union's Seventh Framework Program (FP7/2007-2013) and EFPIA companies' in-kind contribution. We thank Pfizer Ltd for providing d4T and gabapentin. (Huang et al., 2013)

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