Cushioned on the way up, controlled on the way down during resuscitative endovascular balloon occlusion of the aorta (REBOA): investigating a novel compliant balloon design for optimizing safe overinflation combined with partial REBOA ability

Adam Power, 1 Asha Parekh, 2 Neil Parry, 1 Laura J Moore 3

ABSTRACT

Background There are a variety of devices capable of performing resuscitative endovascular balloon occlusion of the aorta (REBOA), with most containing compliant balloon material. While compliant material is ideal for balloon inflation due to its "cushioning" effect, it can be problematic to "control" during deflation. The COBRA-OS (Control Of Bleeding, Resuscitation, Arterial Occlusion System) was designed to optimize inflation and deflation of its compliant balloon and was tested in vitro and in vivo with respect to its overinflation and partial REBOA abilities.

Methods For overinflation, the COBRA-OS was inflated in three differently sized inner diameter (ID) vinyl tubes until balloon rupture. It was then overinflated in six harvested swine aortas and in all three REBOA zones of three anesthetized swine. For partial REBOA, the COBRA-OS underwent incremental deflation in a pulsatile benchtop aortic model and in zone 1 of three anesthetized swine.

Results For overinflation, compared with the known aortic rupture threshold of 4 atm, the COBRA-OS exceeded this value in only the smallest of the vinyl tubes: 8 mm ID tube, 6.5 atm; 9.5 mm ID tube, 3.5 atm; 13 mm ID tube, 1.5 atm. It also demonstrated greater than 500% overinflation ability without aortic damage in vitro and caused no aortic damage when inflated to maximum inflation volume in vivo. For partial REBOA, the COBRA-OS was able to provide a titration window of between 3 mL and 4 mL in both the pulsatile vascular model (3.4±0.12 mL) and anesthetized swine (3.8±0.35 mL).

Discussion The COBRA-OS demonstrated the ability to have a cushioning effect during inflation combined with titration control on deflation in vitro and in vivo. This study suggests that despite its balloon compliance, both safe overinflation and partial REBOA can be successfully achieved with the COBRA-OS.

Level of evidence Basic science.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The balloon segments of most resuscitative endovascular balloon occlusion of the aorta (REBOA) devices are made of a compliant material that is meant to help decrease the rare but potentially lethal risk of aortic damage when occluding the aorta compared with semipliant or non-compliant balloons.

⇒ Although compliant material is ideal for balloon inflation due to its "cushioning" effect, it can be problematic to "control" during deflation due to the difficulty of titrating flow past the balloon and therefore limits partial REBOA (p-REBOA) applications.

WHAT THIS STUDY ADDS

⇒ The COBRA-OS (Control Of Bleeding, Resuscitation, Arterial Occlusion System) is a novel 4 French REBOA device that has a cushioning effect during inflation combined with titration control on deflation.

⇒ This study suggests that despite its balloon compliance, both safe overinflation and p-REBOA can be successfully achieved with the COBRA-OS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study suggests that the COBRA-OS demonstrates safety if accidentally overinflated during REBOA and can be used for p-REBOA applications.

BACKGROUND

Endovascular resuscitation and trauma management techniques1 continue to evolve and resuscitative endovascular balloon occlusion of the aorta (REBOA) is one of the tools available. REBOA provides temporary aortic control in non-compressible torso hemorrhage and allows time for definitive surgical hemostasis. There are a variety of REBOA devices capable of achieving aortic occlusion, each with its own advantages and disadvantages.2 The balloon segments of most of these devices are made of a compliant material that is meant to help decrease the rare but potentially lethal risk of aortic damage when occluding the aorta compared with semipliant or non-compliant balloons.3 While compliant material is ideal for balloon inflation due to its "cushioning" effect, it can be problematic to "control" during deflation due to the difficulty of titrating flow past the balloon. This can lead to significant hypotension on deflation and can limit partial REBOA (p-REBOA) applications. Because of the limitations of traditional compliant
baloons, there has been recent emergence of p-REBOA-specific
devices incorporating semicircular balloon materials.4,5

The COBRA-OS (Control Of Bleeding, Resuscitation, Arterial
Occlusion System) (Front Line Medical Technologies, London,
Ontario, Canada) is a REBOA device that currently has Food and
Drug Administration clearance and Health Canada approval.
Apart from having the lowest profile of any commercially avail-
able REBOA device at 4 French (Fr), the compliant balloon
segment of the device was specifically designed to have even
safer overinflation ability than traditional compliant baloons and
enable more control on deflation for p-REBOA applications.
This descriptive study had the dual purpose of investigating how
well the COBRA-OS provides a “cushion” during inflation and
conversely “control” on deflation. The aim for the “cushion”
portion of the study was to determine device balloon character-
istics (internal pressure, footprint lengthening, circumferential
stretch ratio) and the propensity to cause aortic tissue damage
during varying degrees of severe overinflation. For the “control”
portion of the study, the aim was to determine how controlled
the COBRA-OS was with incremental deflation from full occlu-
sion to determine the applicability to partial aortic occlusion
techniques.

METHODS
This study conforms with the ARRIVE (Animal Research:
Reporting of In Vivo Experiments) guidelines and a complete
checklist has been uploaded as online supplemental digital
content 1. In vitro experimentation was performed prior to
animal study initiation to ensure that there was adequate ratio-
nale to proceed with in vivo experiments. A swine model
was chosen for the in vivo portion of the study because the model has
been used extensively for aortic occlusion studies, resulting in
a large volume of data on the vascular response and its correlation
to human vascular response. The minimum number of animals
possible was used in this study and did not duplicate any previous
work. Due to this being a feasibility and mainly descriptive study,
no power calculations were performed to determine the sample
size and no randomization or blinding occurred. Numerical data
are presented as mean with SD. The protocol (study no: 3101-
554N) was reviewed and approved by the testing facility’s Insti-
tutional Animal Care and Use Committee. The review ensured
compliance with the Canadian Council on Animal Care regu-
lations. The testing facility is accredited by the AAALAC (Associa-
tion for Assessment and Accreditation of Laboratory Animal
Care) International and the Canadian Council on Animal Care.

In vivo experiments were performed on three female
domestic farm pigs Sus scrofa (Landrace-Yorkshire), with weights
ranging from 78.4 kg to 93.0 kg. The animals were anesthetized
with ketamine, azaperone, and atropine administered intra-
muscularly. An intravenous catheter was placed in an appro-
priate vein and anesthesia induction for tracheal intubation was
achieved with propofol intravenously. On induction of anes-
thesia, the animals were intubated and supported with passive
ventilation. Isoflurane in oxygen was administered to maintain
a surgical plane of anesthesia and intravenous fluid therapy was
initiated and maintained throughout the procedure. Once anes-
thetized, the following intra-arterial access sites were gained on
each of the animals: 7 Fr sheath in the left carotid artery for
proximal arterial pressure monitoring, 7 Fr sheath in the left
femoral artery for distal arterial pressure monitoring and angio-
graphy, and a 4 Fr sheath in the right femoral artery for aortic
occlusion with the COBRA-OS. Fluoroscopic guidance and angi-
graphy were used to assess anatomy and identify the proper
location for device positioning and aortic diameters. Proximal
and distal mean arterial pressures (MAPs) were recorded every
5 seconds using an Emka data acquisition unit throughout the
duration of the study. p-REBOA experiments were performed
first, followed by overinflation experiments, and once complete
the animals were killed and sent for necropsy.

Cushioning: overinflation ability
In vitro
The pressure that can be generated within a balloon segment
during inflation is directly proportional to blood vessel rupture
risk, and therefore the COBRA-OS was inflated until failure in
stiff vinyl tubes to investigate the maximum pressure that could
be produced inside the baloons. The COBRA-OS was inflated
until the balloon segment made circumferential wall contact and
then was serially inflated by 1 mL increments in three differently
sized inner diameter (ID) vinyl tubes (7.94 mm, 9.53 mm, 12.7
mm) until balloon rupture occurred. A digital inflation device
(Blue Diamond Digital Inflation Device, Merit Medical, UT) was
used to inflate the baloons and record the inflation pressure at
each inflation step as well as the maximum pressure just prior
to rupture. Balloon footprint length was also measured at each
step.

To further investigate the overinflation ability of the
COBRA-OS, fresh thoracic aortic tissue from six female domestic
farm pigs (Landrace-Yorkshire) weighing approximately 70 kg
were obtained from a local commercial pig farm immediately
after killing the animal. Institutional approval was not required
as this was a postmortem study. The COBRA-OS devices were
inflated initially to 13 mL (maximum as per Instructions For
Use [IFU], 25 mm diameter) and then serially inflated by 10
mL increments until the balloon or aorta ruptured. At baseline
and each inflation step, the measured aortic diameter and the
length of the balloon were recorded using a Vernier caliper. The
measured aortic diameters were compared with baseline aortic
diameters to calculate a circumferential stretch ratio.

In vivo
To investigate the safety of inflation to maximum IFU volume in
vivo and to further the idea of “fixed-volume aortic occlusion
(FVAO)”, swine with aortas intentionally undersized compared
with humans were used. The COBRA-OS was inflated until
aortic occlusion was confirmed and then intentionally overin-
flated to 13 mL (maximum as per IFU, 25 mm diameter) in zone
1 (mid-descending thoracic aorta), zone 2 (renal arteries), and
zone 3 (aortic bifurcation) in all three animals. Angiograms were
performed at each of these zones to measure aortic diameters.
Animals were killed and a limited necropsy was performed for
gross examination of the treatment sites for evidence of damage.
Anatomic markers, treatment angiographic images, and proce-
dural notes were used to help identify the treated vessel sites as
accurately as possible.

Control: p-REBOA ability
In vitro
The COBRA-OS was tested on a closed-loop, three dimensional-
printed vascular model with a pulsatile pump. Pressures above
and below the balloon were measured using a digital pres-
sure monitor (Compass device, Centurion Medical Products,
Memphis, TN). The device was inflated until occlusion of the
aorta (21 mm diameter) and then serially deflated by 0.2 mL
increments every 30 seconds until pressures above and below
the balloon equalized. This was repeated two further times to confirm the results.

In vivo
The COBRA-OS was placed in zone 1 (mid-descending thoracic aorta) and inflated until aortic occlusion was confirmed in all three animals. The device was then serially deflated by 0.2 mL increments every 30 seconds until pressures above and below the balloon equalized. Fluoroscopy was used to visualize the balloon on deflation to monitor its shape.

RESULTS

Cushioning: overinflation ability

In vitro
In the 7.94 mm ID tube, the COBRA-OS generated a maximal internal pressure of 6.5 atm, while in the 9.53 mm and 12.7 mm ID tubes the devices generated maximal internal pressures of 3.5 atm and 1.5 atm, respectively (table 1, figure 1). The footprint lengths at rupture ranged from 90 mm to 110 mm.

The mean diameter of the harvested thoracic aortas was 21±0.10 mm and all balloons ruptured before causing gross intimal damage. The mean balloon overinflation volume at rupture for the COBRA-OS was 86±6.2 mL, which represents a 562% increase over the IFU volume (13 mL), and the mean footprint length at rupture was 130±14 mm (figure 2). The mean circumferential stretch ratio at balloon rupture was 1.53±0.04.

In vivo
The mean weight of the animals used in the study was 84±6.6 kg. In zone 1, the mean aortic diameter was 15.0±0.4 mm, the mean occlusion volume was 6.0±1.0 mL, and the mean overinflation percentage above maximum IFU (13 mL, 25 mm diameter) was 117±37%. In zone 2, the mean aortic diameter was 14.4±0.4 mm, the mean occlusion volume was 5.4±0.6 mL, and the mean overinflation percentage was 141±24%. In zone 3, the mean aortic diameter was 12.0±1.1 mm, the mean occlusion volume was 5.0±0 mL, and the mean overinflation percentage was 160±0%. On necropsy, there was no evidence of intimal damage or thrombus in any treatment zone.

Control: p-REBOA ability

In vitro using the closed-loop pulsatile model, proximal and distal pressures returned to baseline over a mean deflation volume of 3.4±0.12 mL for the COBRA-OS. Subsequently, in vivo, proximal and distal pressures equalized in a linear fashion over a mean deflation volume of 3.8±0.35 mL (figure 3). The COBRA-OS device maintained its overall shape throughout deflation (figure 4).

DISCUSSION

The COBRA-OS is a 4 Fr REBOA device that was designed to optimize inflation and deflation of its compliant balloon. These in vitro and in vivo studies show that the COBRA-OS has safe overinflation ability and a linear, relatively wide window of titration control for p-REBOA applications.

Aortic rupture during REBOA is a rare but potentially lethal complication. Although infrequent with most current generation...
REBOA devices, there are a variety of circumstances whereby additional overinflation ability might prove useful. REBOA in pediatric trauma is currently being investigated, and since pediatric aortic sizes are smaller and more variable depending on age, balloon overinflation may occur, predisposing patients to aortic injury. The issue of variable blood vessel sizes also occurs when performing balloon occlusion for proximal control in arteries other than the aorta, such as in the axillary/subclavian artery or iliac artery, before vascular repair. The iliac artery itself can also be iatrogenically injured during zone 3 REBOA due to migration of the balloon caudally, and the additional overinflation ability of the COBRA-OS has previously been found to help prevent this complication in an animal model. Further, safe overinflation may be useful in patients that are severely hypotensive when performing proximal and distal occlusion confirmation techniques may not be accurate and during REBOA with an overzealous inexperienced user who might inadvertently overinflate.

In our study, inflation of devices in the vinyl tubes showed that as the tube diameter increased, the pressure generated by the devices decreased and the balloons ruptured at lower internal pressures. The quoted rupture pressure of the aortic wall is 4 atm and the COBRA-OS went beyond 4 atm only in the smallest diameter tube (7.94 mm ID), which is much smaller than typical adult human aortic diameters. Circumferential stretch ratio has been used to determine aortic failure in multiple previous studies. The ER-REBOA (Prytime Medical, TX) was previously investigated for rupture risk and the authors found the circumferential stretch ratio at aortic failure to be 1.8, which aligns with other studies that report 1.7. The ER-REBOA was only inflated to 24 mL (maximum as per the manufacturer’s IFU) and three ruptures occurred in 14 specimens. In contrast, the COBRA-OS had no ruptures in harvested aortas and never generated a circumferential stretch ratio greater than 1.6 despite greater than 500% overinflation. This overinflation ability was further confirmed in vivo whereby significant overinflation to maximum IFU inflation volume resulted in no vessel damage or rupture of the balloons. The mean aortic diameter was only 15 mm in zone 1, and even smaller in the other zones, which represented an undersized human aorta (average diameter of 22–23 mm). This furthers the possibility of having a “fixed-volume aortic occlusion” device that allows significant overinflation to simplify procedures for providers that may have less experience or skill. Thus, monitoring proximal or distal pressures to confirm occlusion would not be needed and instead providers would inflate to a set single volume (13 mL) in all patients.

The “cushioning” effect of the COBRA-OS is mainly due to its unique “Safety Shoulder Reservoir.” The compliant balloon material extends beyond the balloon footprint with a proximal tapered segment (shoulder). As the COBRA-OS inflates and meets the wall of the vessel and continues inflating, the additional volume and pressure is offloaded into the reservoir, resulting in lengthening of the balloon caudally as opposed to having the balloon continue to increase in diameter if accidental overinflation occurs (figure 5). It is important to note that when used as per IFU, the footprint of the COBRA-OS remains at 4 cm to 6 cm during full aortic occlusion. The balloon only grows in length if a user does not monitor when cessation of distal flow is achieved and instead inadvertently continues to inflate.

p-REBOA is a newer technique that is being investigated to potentially mitigate the harmful effects of prolonged aortic occlusion, allowing for hypertension above the balloon, some degree of partial flow past the balloon to perfuse organs, and permissive hypotension below the balloon to help limit hemorrhage. As mentioned previously, traditional compliant balloons have difficulty achieving this for a variety of reasons, although many authors have reported on p-REBOA using older generation REBOA devices. The ideal degree of “fine tuning” is completely unknown and most studies have targeted a single distal MAP (20–40 mm Hg) as opposed to continuously manipulating the amount of partial flow past the balloon according to physiological needs. Therefore, a large titration window is likely not required if the p-REBOA device can hit and maintain a set target distal pressure range. Additionally, it is unknown if intermittent REBOA is a useful alternative strategy to p-REBOA, where balloon titration ability would not be needed.
One of the main concerns with current REBOA techniques is the use of a 7 Fr sheath, which can limit the outflow to a patient’s limb and may be further exacerbated over a planned longer p-REBOA period. Despite the possible benefits of partial occlusion with a 7 Fr sheath, these may have to be balanced with an elevated risk of limb complications due to the outflow obstruction. Reported limb complications that happen after complete REBOA using a 7 Fr sheath primarily affect the access site limb, as opposed to both limbs, implying that this is more related to the unilateral sheath combined with aortic occlusion as opposed to the aortic occlusion itself. A recent large retrospective study by Laverty et al and the AORTA (Aortic Occlusion for Resuscitation in Trauma and Acute Care Surgery) investigators reported 24 of 352 (7%) arterial access-related limb ischemic complications using the 7 Fr ER-REBOA (Prytime Medical). The use of a 4 Fr REBOA device with p-REBOA abilities, such as the COBRA-OS, may help to mitigate these complications in both partial and complete REBOA.

Our in vitro and in vivo studies revealed that the COBRA-OS has a window of p-REBOA titration ability between 3 mL and 4 mL of volume removed from the balloon. Functionally speaking, it was easy to take small 0.2 mL aliquots from the balloon with the supplied 10 mL syringe (0.2 mL gradations) to achieve this. Removing small aliquots has been difficult to achieve in the past with previous devices because all other REBOA devices before the COBRA-OS had large inflation lumens, and when you opened the stopcock to deflate, the inflation fluid quickly drained from the device, even without drawing back on the syringe, making precise deflation unachievable. In response to this, DuBose described a technique that used a 3 mL syringe attached to a three-way stopcock to help precisely control deflation over 0.1 mL increments. A previous animal study investigated incremental deflation of the ER-REBOA following 60 minutes of full occlusion in a hemorrhagic model and found that rapid return of flow occurred over a single 0.5 mL step. The single inflation lumen of the COBRA-OS is much smaller than in other REBOA devices, which allows small aliquots of inflation medium to be removed from the balloon more easily compared with other devices. It is worth noting that the minimum time it takes once you open the stopcock and withdraw on the syringe to go from full occlusion to full equalization of proximal and distal blood pressures is probably a more useful measure of a device’s p-REBOA ability as opposed to titration windows. For an average diameter human aorta, this happens very quickly with other REBOA devices, while this cannot happen any faster than 10 to 15 seconds with the COBRA-OS (see online supplemental digital content 2).

The balloon material of the COBRA-OS is compliant, and the offset shape is designed to counteract the “tear-drop” shape that normally forms when deflating the balloon of older generation REBOA devices. This tear-drop effect makes most spherical balloons act more like an “on/off switch” as opposed to the preferred “dimmer switch.” Additionally, the COBRA-OS balloon has mechanical properties that allow it to maintain its overall shape throughout inflation and deflation cycles as seen in vivo from fluoroscopy images and is demonstrated in online supplemental digital content 3. This design feature was implemented so that once a target distal pressure was reached, there would be less balloon volume manipulation required to maintain the desired distal MAP.

In clinical practice, p-REBOA with the COBRA-OS can be performed by first transcending the sidewall of the supplied 4 Fr sheath for distal MAP measurements. With full aortic occlusion, the MAP is close to 0 and no pulsatile wave form is seen. The stopcock is then opened, and withdrawal of the syringe is performed until a pulsatile wave form first appears. A preferred distal MAP can then be adjusted by removing 0.2 mL to 0.4 mL aliquots until the target MAP is achieved. It is important to note that for efficiency these small incremental inflations or deflations are only done after the wave form first appears and not from full occlusion as was done in this study.

There are several limitations to the current study. The experiments were mainly performed to investigate the function and performance of the COBRA-OS and therefore highly controlled in vitro and in vivo models were used. Future in vivo studies should investigate these properties in a hemorrhagic animal model with prolonged occlusion to better simulate clinical use environments. Also, flow was not measured in vivo as previously done in other studies. Proximal and distal pressures alone were used in this study because it is the only clinically available option currently to estimate the degree of p-REBOA. With regard to aortic rupture risk, swine aorta is reasonable for comparison with human aorta but the walls are known to be thicker and therefore direct correlation to humans is cautioned.

CONCLUSION

The COBRA-OS has demonstrated the ability to have a cushioning effect during inflation combined with titration control on deflation in vitro and in vivo. This study suggests that despite its balloon compliance, both safe overinflation and p-REBOA can be successfully achieved with the COBRA-OS. These features may be useful when performing REBOA clinically.

Acknowledgements The authors would like to thank Seema Gogna for her assistance in helping with the animal lab research.

Contributors APo and APa were involved in study design. APo and APa were involved in data collection. All authors were involved in data analysis, data interpretation and critical revisions. The first draft of the article was written by APo and is the author responsible for the overall content as the guarantor.

Funding This study was funded by Front Line Medical Technologies (paid for the animal study expenses).

Competing interests APo and APa are cofounders and have an equity stake in Front Line Medical Technologies. LJM is Chair of the Scientific Advisory Board, has an equity stake, and receives consulting fees from Front Line Medical Technologies. NP is on the Scientific Advisory Board and owns shares in the company.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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REFERENCES


doi:10.1136/tsaco-2022-000948


Not commissioned; externally peer reviewed.


The ARRIVE guidelines 2.0: author checklist

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

<table>
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<th>Item</th>
<th>Recommendation</th>
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<td>Study design</td>
<td>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).</td>
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<td>Sample size</td>
<td>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.</td>
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<td>Inclusion and exclusion criteria</td>
<td>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.</td>
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<td>Randomisation</td>
<td>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.</td>
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<td>Blinding</td>
<td>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).</td>
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<td>Outcome measures</td>
<td>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.</td>
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<td>Statistical methods</td>
<td>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.</td>
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<td>Experimental animals</td>
<td>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.</td>
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<td>Experimental procedures</td>
<td>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).</td>
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<td>Results</td>
<td>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.</td>
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# 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.

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<td>Abstract</td>
<td>11 Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.</td>
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<td>Background</td>
<td>12 a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.</td>
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<td>b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.</td>
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<td>Objectives</td>
<td>13 Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.</td>
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<td>Ethical statement</td>
<td>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.</td>
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<td>15 Provide details of housing and husbandry conditions, including any environmental enrichment.</td>
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<td>Animal care and monitoring</td>
<td>16 a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.</td>
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<td>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.</td>
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<td>Interpretation/scientific</td>
<td>17 a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.</td>
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<tr>
<td>implications</td>
<td>b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.</td>
<td></td>
</tr>
<tr>
<td>Generalisability/translation</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>Protocol registration</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Data access</td>
<td>20 Provide a statement describing if and where study data are available.</td>
<td></td>
</tr>
<tr>
<td>Declaration of interests</td>
<td>21 a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.</td>
<td></td>
</tr>
</tbody>
</table>

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