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Citric Acid Water Restriction

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How to prepare and carry out citric acid water restriction for motivating mice to engage in behaviour.

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Mouse, Behaviour, Water Restriction, Citric Acid

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Any mice dropping to below 80% of their pre-CA baseline weight must be returned to *ad lib* water (see Duguid Lab PPL for regulations)

Baseline Weights 3d

1 Handle animals and weigh for 3-5 days to establish a mean baseline weight prior to introduction of the CA water

Preparing 2% CA Water 15m

2 Prepare the following:

- 2 bottles
- Citric acid crystals

- Sterile Gloves or weighing boats
- Scales

2.1 Empty one of the water bottles, place it on the scales and tare/zero

2.2 Pour the water from the other bottle into the one on the scales, record the weight of water

2.3 Calculate 2% weight of the water, this is the weight of citric acid crystals you need

e.g. 300g of water, requires 6g of citric acid (300*0.02 = 6)

2.4 Use the plastic side of the packet containing the sterile gloves or a weighing boat to weigh out the citric acid crystals

2.5 Add crystals to the bottle of water, put on the lid/spout and shake with finger covering the opening until dissolved

2.6 Label the bottle on 2 sides as "2% Citric Acid". Add a 'DO NOT REMOVE' label to the base of the bottle

Starting water restriction 5m

3 Replace normal water bottle with 2% CA bottle, perform behaviour with 10% sucrose as a reward

3.1 Weigh animals daily to ensure they do not drop below 80% of their baseline weight

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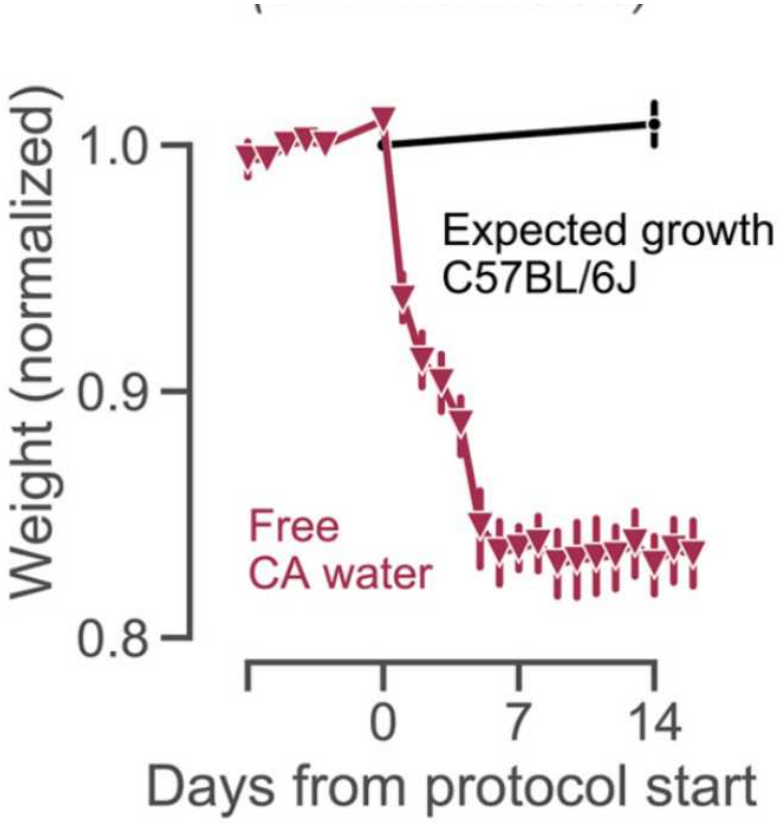

Plot from Urai 2021 (https://www.eneuro.org/content/8/1/ENEURO.0230-20.2020) showing typical progression of weight loss for mice during CA water restriction

3.2 If any animal drops below 80% of their baseline weight, return to *ad lib* water per the PPL

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