

Video Article

An Alternant Method to the Traditional NASA Hindlimb Unloading Model in Mice

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Abstract

The Morey-Holton hindlimb unloading (HU) method is a widely accepted National Aeronautics and Space Administration (NASA) ground-based model for studying disuse-atrophy in rodents⁴⁻⁶. Our study evaluated an alternant method to the gold-standard Morey-Holton HU tail-traction technique in mice. Fifty-four female mice (4-8 mo.) were HU for 14 days (n=34) or 28 days (n=20). Recovery from HU was assessed after 3 days of normal cage ambulation following HU (n=22). Aged matched mice (n=76) served as weight-bearing controls.

Prior to HU a tail ring was formed with a 2-0 sterile surgical steel wire that was passed through the 5th, 6th, or 7th inter-vertebral disc space and shaped into a ring from which the mice were suspended. Vertebral location for the tail-ring was selected to appropriately balance animal body weight without interfering with defecation.

We determined the success of this novel HU technique by assessing body weight before and after HU, degree of soleus atrophy, and adrenal mass following HU. Body weight of the mice prior to HU (24.3 ± 2.9 g) did not significantly decline immediately after 14d of HU (22.7 ± 1.9 g), 28d of HU (21.3 ± 2.1 g) or after 3 days recovery (24.0 ± 1.8 g). Soleus muscle mass significantly declined (-39.1%, and -46.6%) following HU for 14 days and 28 days respectively ($p < 0.001$). Following 3 days of recovery soleus mass significantly increased to 74% of control values. Adrenal weights of HU mice were not different compared to control mice.

The success of our novel HU method is evidenced by the maintenance of animal body weight, comparable adrenal gland weights, and soleus atrophy following HU, corresponding to expected literature values^{2,7,8}. The primary advantages of this HU method include: 1) ease of tail examination during suspension; 2) decreased likelihood of cyanotic, inflamed, and/or necrotic tails frequently observed with tail-taping and HU; 3) no possibility of mice chewing the traction tape and coming out of the suspension apparatus; and 4) rapid recovery and normal cage activity immediately after HU.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2467/>

Protocol

Pre-Surgery Instruments / Materials

- Wire cutters/pliers
- Gauze 2 x 2" pads
- Heating pad
- Silver nitrate sticks
- Sterile surgical gloves
- Isoflurane inhalation anesthetic
- Chlorhexidine Gluconate 4% (Antiseptic surgical scrub)
- Sterile 2-0 surgical steel suture
- 25 X 5/8 A gauge needle (Autoclaved)
- Hemostat (Autoclaved)
- Surgical steel suture

Surgery Procedure

1. Create a sterile field. Follow sterile procedure.
2. Anesthetize mouse with 2 - 3% isoflurane.
3. Place mouse on the heating pad.

4. Scrub tail with Chlorhexidine solution.
5. Locate 5, 6, or 7th intervertebral space (between two bony bumps on tail, start counting from end of body hair, at the base of tail).
6. Use Hemostat and 25G needle to make a pilot hole between two vertebrae, into the intervertebral space.
7. Use the wire cutters to cut the steel suture 10 cm long.
8. Remove needle and insert steel suture through hole, 5 cm of suture on either end of tail.
9. Each side of the tail should have + 5 cm of suture.
10. Place thumb tip onto tail.
11. Use your free hand and wind the steel suture onto the tip of your thumb and twist the ends to form a big loop. The wound or circular part should be + 3 mm long.
12. Remove your thumb.
13. Clamp one strand of suture with the hemostat and bend it into a smaller loop, the end of which is attached as closely as possible to the twist on the original large loop.
14. Cut off excess wire, and bend the twisted wire back.
15. If there is any excess bleeding, you can stop it with the silver nitrate sticks.
16. Turn off the isoflurane and place mouse in a single house cage to recover for 5-7 days prior to hindlimb unloading. The tail rings get entangled if more than one mouse is housed together.

1. Hindlimb Unloading Procedure

Materials

- Size 10-24 X 4 machine screws, one per animal.
- Nylon lock nut size 10 - 24, one per animal.
- Wing nut size 10 X 24, two per animal.
- Washer size ¼ X 1, two per animal.
- Fishing swivel with snap hook size #6, 1-11/16", and one per animal.
- Metal sewing machine bobbin. One per animal.
- Small paper clip for S-shaped hook, one per animal
- Pliers.
- 2 x 2" gauze.
- 2" black paper binder clamps.
- Duct tape/ lab tape.
- 1-3/8" Plated slotted steel bar that extends slightly beyond the full length of the cage
- 50cm X 40cm X 20cm cage (rat cage).
- Water Bottle with bended tube.
- Stainless Steel wire, U-shaped to support water bottle.

Cage Setup

1. Lay the 1-3/8" Plated slotted steel bar over the top of the cage, and tape it temporally down with duct tape.
2. Screw 1 wing nut to the machine screw, insert a washer, and insert the machine screw trough the slotted steel plate ± 15 cm from the edge of the cage.
3. Add another washer and wing nut. Tighten both wing nuts, but do not fully tighten, as it will be necessary to do adjustments later.
4. Insert the machine screw through the center hole of the bobbin.
5. To prevent the bobbin from falling off, screw the nylon lock nut to the end of the machine screw. The bobbin should swivel freely.
6. Insert the snap hook from the fishing swivel through the lower part of the bobbin.
7. Use pliers to form an S-shaped hook from the paperclip. (Cut 2cm from the paper clip to form a hook).
8. Insert one end of the S-shaped hook through the hole of the fishing swivel and pinch it shut with the pliers.
9. Place the U-shaped wire, parallel to the machine screw, on the inside of the cage side, and bend the ends back to support a full water bottle.
10. Insert the water bottle into the U-shaped wire, with the spicket facing down, but not touching the bottom.
11. Bend the 2" binder clamp open, so that they cannot shut close. Just enough tension to hold 3 to 4 pieces of food in it without it falling out. This is preventing the force of the clamp from crushing the food, or clamping down on the mouse nose.
12. Place 3 to 4 pieces of rodent chow into the binder clamp.

Hindlimb Unloading of Mouse

1. Insert the open end of the S-shaped hook through the smaller ring of the tail ring.
2. Adjust the mouse height by tightening or loosening the lower wing nut on the machine screw. The lower extremities should not be weight bearing. Once the optimal height is achieved tighten the upper wing nut.
3. Reposition the water bottle, and U-shaped wire so that the mouse cannot climb onto the spicket with its hindlimbs. Secure the water bottle with Duct tape to the side of the cage.
4. Position the binder clamp with food on the bottom of the cage so that the mouse cannot climb onto it; tape it down with duct tape.
5. Repeat the same procedure if more than one animal is hindlimb unloaded on the other side of the cage. House two animals per cage; space the animals so that they can almost touch each other.
6. Use the 2 X 2 gauze to loosely wrap the tail of the mouse onto the fish swivel. Use a small piece of tape to wrap around the gauze, securing the gauze to the tail. This step is important to prevent the tail from drooping downward, leading to blood pooling in the tip of the tail and that tail becoming necrotic.
7. Add a small amount of cob bedding to the cage. Do not add too much bedding otherwise the mouse will from a mound of bedding and attempt to load hindlimbs on bedding.

2. Representative Results:

To establish the success of the tail-ring HU technique we measured animal body weight before the tail ring surgery, and prior to and following HU and recovery; soleus muscle mass; and adrenal mass in all groups.

Body weights of the mice throughout the experiment are shown in Figure 1. Body weights of the mice prior to the tail ring surgery ($24.7 \pm 3.5\text{g}$) were not significantly different after 5 days of recovery from the tail ring surgery ($24.3 \pm 2.9\text{g}$) prior to HU. Nor did body weight significantly decline immediately after 14 days of HU ($22.7 \pm 1.9\text{g}$), 28 days of HU ($21.3 \pm 2.1\text{g}$) or after 3 days recovery from HU ($24.0 \pm 1.8\text{g}$). Although HU for 14 and 28 days resulted in a small (-6.8% and 12%) but non-significant loss in body-mass, the mice fully recovered to their pre-HU body weights after 3 days of recovery. Food consumption for ambulatory control mice and HU mice is shown in Figure 3. Average daily food intake was not different in HU mice over 28 days compared to controls.

We observed significant soleus muscle atrophy following HU for 14 and 28 days (-39.1%, and -46.6%) indicating an effective HU procedure (Figure 2). Furthermore this degree of atrophy is consistent with the expected literature values for 2-4 weeks of HU^{2,7,8}. Following 3 days of recovery from HU soleus mass significantly increased to 74% of control values. Soleus mass in the recovery group was significantly greater than the both HU groups, but still significantly less than ambulatory control animals.

Adrenal weights were measured as an indicator of the animal stress; both the left and right adrenal glands were excised and weighed. The average weights were comparable for control and HU mice in all groups (Figure 4).

Previously in rats we have shown that the success rate for the number of animals at the beginning of HU compared to the number of animals that completed HU without coming out of suspension apparatus (due to animals chewing through the tail-traction tape) was 10 out of 12 using the Morey-Holton technique¹. None of the mice ($n=54$) in this study ever came down from suspension prior to the completion of the HU period. We did however, have one mouse that developed a severely inflamed tail. When inflammation became apparent, we removed the tail ring to allow the inflamed area to heal, and subsequently placed another tail-ring to complete the duration of HU.

In the past when we have used the Morey-Holton method of HU, we typically eliminate 1-2 mice/group because they come out of the suspension apparatus. With the tail-ring method there were no episodes of mice inadvertently coming out of the HU apparatus during the period of HU for 14 or 28 days ($n=54$ mice).

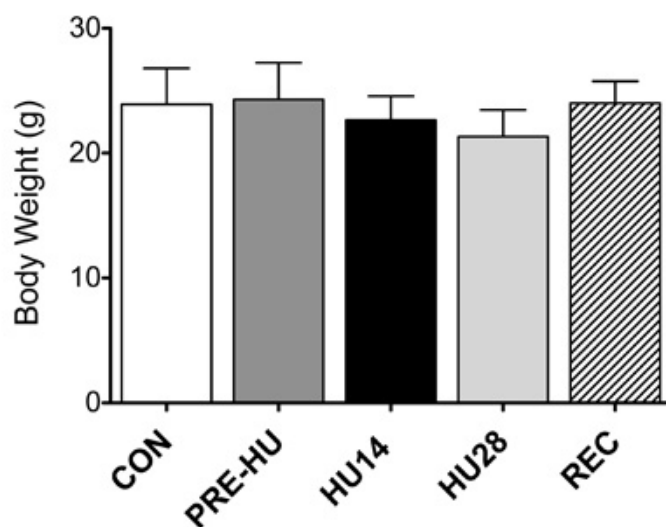


Figure 1. Body Weight Changes (Mean \pm SD). Abbreviations: CON: weight-bearing control mice ($n=76$); Pre-HU: all mice prior to HU ($n=54$); HU14: mice hindlimb unloaded for 14 days ($n=34$); HU28: mice hindlimb unloaded for 28 days ($n=20$); REC: hindlimb unloaded mice allowed to cage recover for 3 days ($n=22$). No significant differences in body weight were observed between groups.

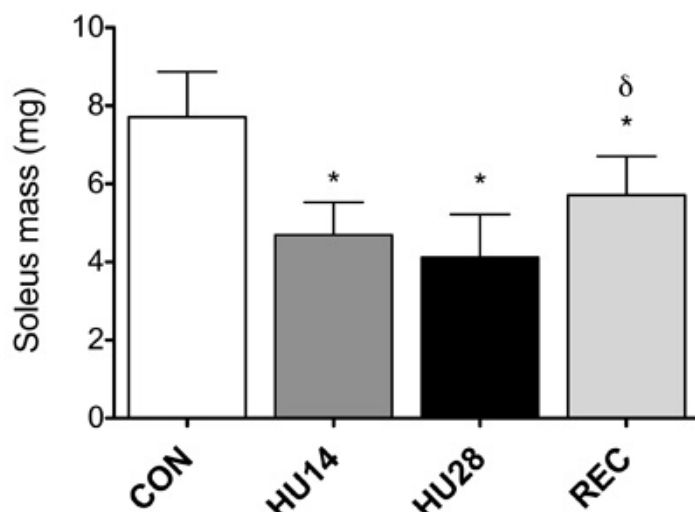


Figure 2. Soleus Muscle Atrophy (Mean \pm SD). Abbreviations: CON: weight-bearing control mice (n=76); HU14: mice hindlimb unloaded for 14 days (n=34); HU28: mice hindlimb unloaded for 28 days (n=20); and REC: hindlimb unloaded mice allowed to cage recover for 3 days (n=22). *Significantly different ($p < 0.001$) than weight-bearing control (CON). δ Significantly different ($p < 0.01$) than both HU groups.

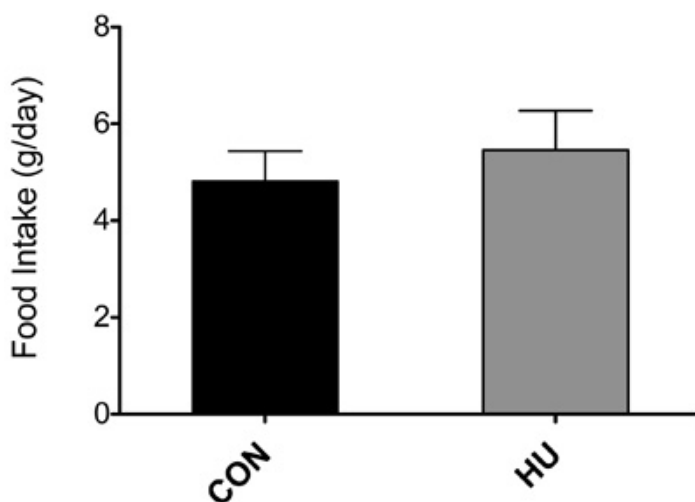


Figure 3. Food Consumption (Mean \pm SD). Average daily food intake (g/day) during weight-bearing and hindlimb unloading. Abbreviations: weight-bearing control mice (n=76); HU: mice hindlimb unloaded 14 - 28 days. No significant differences between CON and HU groups were observed.

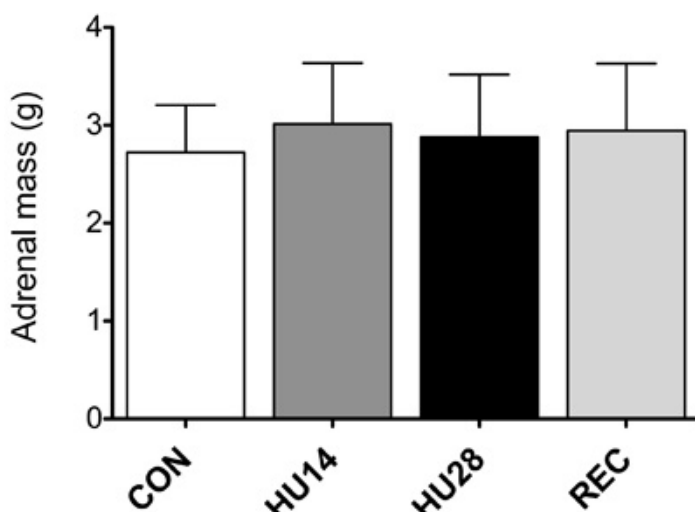


Figure 4. Adrenal Mass (Mean \pm SD). Abbreviations: CON: weight-bearing control mice (n=76); HU14: mice hindlimb unloaded for 14 days (n=34); HU28: mice hindlimb unloaded for 28 days (n=20); and REC: hindlimb unloaded mice allowed to cage recover for 3 days (n=22). No significant differences were observed between the groups.

Discussion

We evaluated an alternative method of hindlimb unloading mice, which we determined to be highly successful. The tail-ring method as described here was used for long-term hindlimb suspension, and to study recovery following HU. Under each of these conditions, there was no evidence of failure to adapt as evidenced by stable food intake, body weights, and adrenal weights of the mice. This tail-ring HU technique is easy to perform and can readily be incorporated with very little surgical practice.

There are several advantages of tail-ring method compared to the traditional Morey-Holton^{5,6} method. First, the skin and ring implantation site on that tail are always visible, so if problems arise it is evident immediately, not just at the end of the suspension period. Second, animals are not able to get out of the suspension apparatus at any time during HU, obviating the need to make a determination of whether to keep a mouse or not in the data set. Furthermore, the mice do not bother the ring apparatus and when they are released from HU for recovery, they do not tamper with their tails, allowing for a seamless recovery phase following HU. Finally, hindlimb unloaded mice with the tail-ring method maintain their body weight better than mice hindlimb unloaded by the Morey-Holton tail-traction technique. In our hands after 14 days of HU we observed a small (-6.8%) but non-significant loss in body-mass. Whereas Ingalls *et al.* observed a significant -12% decrease in body weight in mice after 14 days of HU by the Morey-Holton method³. Although Ingalls did not report adrenal weights, it appears that our tail-ring method confers less stress on the mouse (adrenal weights in our HU mice are not different than controls) and better maintenance of body weight throughout the hindlimb unloading period.

In summary, a tail ring method of hindlimb unloading was developed and tested on mice HU for 14 and 28 days, and on mice returned to cage recovery for 3 days after HU. On the basis of adrenal weights, body mass and food intake, mice appear to have adapted well to the tail-ring apparatus. The tail-ring hindlimb unloading method is a useful, simple alternative to the traditional Morey-Holton hindlimb unloading technique.

Disclosures

No conflicts of interest declared.

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