**Standard Operation Procedure** (SOP): Treadmill running for Mice

**Introduction**

Exercise parameters such as speed, duration and angle of the treadmill can be strictly controlled to fulfill a variety of experimental protocols and allow a precise exercise load to be set. Mice belonging to an experimental group undergo the same exercise regimen. Control of the parameters facilitates comparison of results. When the environmental and exercise conditions are the same, this will limit bias. Micetreadmill experiments cannot be conducted without an acclimatizing period of 3 to 7 days, during which animals become familiar with the apparatus and can then train for longer periods of time and at higher speeds. Running can induce a certain level of stress in the animal. Continuous supervision by two research personnel is required. Running of 5-6 mice ensures better adherence to experimental protocol. It is essential to use standardized protocols and proper methods to protect mice subjects and collect authentic data fulfilling approved IACUC protocol. It is also crucial to maintain standard conditions throughout the protocol, especially when exercise is performed regularly.

Our lab currently has Exer 3/6 Treadmill. As described in the manufacturer manual: (The Columbus Instruments Exer 3/6 Treadmill is designed for general-purpose animal exercising. The overall running surface is divided into individual lanes by use of clear or opaque lane dividers. By default, the outermost lane dividers are clear to allow the user to view the animal in the outside lanes. All remaining dividers are opaque to provide isolation. The treadmill is capable of exercising from 1 to 3 rats or from 1 to 6 mice simultaneously. The running surface can be inclined up to 25° above the horizontal in 5° steps. An optional Downhill Running Adapter allows the running surface to be declined down to 15° below the horizontal in 5° steps. A clear one-piece lid and hinge cover all of the lanes. The lid and dividers are easily removed for cleaning. The Treadmill Controller allows the user to set the parameters for the treadmill belt speed and optional Electric Stimulus. The treadmill belt speed is adjustable from 0.0 to 102.3 meters per minute (m/min). A toggle switch commands the treadmill belt to “RUN”, “STOP” and “ACCELERATE”, a new feature of the controller. An LCD display reports the current speed setting, treadmill belt status as well as the accumulated distance traveled. The optional Electric Stimulus is a 200 millisecond (ms) pulse of electric current adjustable from 0.34 to 1.60 milliamperes (mA). The pulse is presented 1, 2 or 3 cycles per second (Hertz, Hz). Individual toggle switches associated with each lane on the treadmill controls the application of the Electric Stimulus to the shock grids.

**Objective:**

The objective of this SOP is to describe how the treadmill can be used for the study of rodent exercise. Mice are subjected to an enforced running pattern where speed, duration and elevation (no elevation will be used in the current approved IACUC protocol) can be adjusted and monitored.

**Applicability:**

Rodent exercise using treadmills can be used to study the effect of physical activity on health and disease as exercise can modify the progression of certain diseases. Our lab will be using the treadmill to study the effects of exercise on mechanisms of atherosclerosis and cardiovascular disease.

**A. Preparing for treadmill use:**

The following guidelines must be followed before the animals are introduced to the treadmill:

* Stop all activities that are not related to the work and that may distract the operators from concentrating on the treadmill during the period when animals are on the belt
* Inspect the treadmill and make sure it is clean, operational, and configured correctly before placing the animals on the belt
* At least two operators **must** be present at all times while the treadmill is in use
* All animals in the exercise groups **must** be observed for signs of injury to the toes or feet as well as overall health before being placed on the treadmill. Any animal that shows signs of injury, pain/distress or declining health **must** be removed from the study. The PI and the facility manager must be notified immediately if any animal is removed from the study for any health reasons
* Before the exercise session begins the instrument log and lab notebook **must** be consulted to review the notes from the previous session. The lab notebook will provide pertinent information regarding animal health, including detailed notes on the following:
* Body weight (to be monitored weekly)
* Food and water consumption (monitored weekly)
* Change in mood (too nervous or too sleepy (monitored daily)
* Marked change in the ability to perform the exercise (e.g. more than 3-5 stops per each treadmill trial session)
* Change in social habits
* If there are any concerns not addressed here, consult the instruction manual near the treadmill or contact <insert name>

**B. Animal acclimation to the treadmill**:

Acclimation of the treadmill is important to allow the mice to become familiar with the treadmill and to minimize psychological stress. This should be done for 5–15 minutes per day at least once per day for several days. During this period the electric grid will remain off and the belt motor on but not moving. The mouse should then be familiarized with the sounds and experiences of the moving treadmill by turning the treadmill on at the lowest speed setting and allowing the mouse to walk or run slowly for 5–15 minutes. The shock grid will not be used during the acclimation period.

**1. Warm-up**:

*The length of the warm-up, the initial speed, and the rate of acceleration are dependent on the desired final speed:*

* Start the belt at the slowest speed (1m/min) for 1-2 minutes or as long as needed for the mice to acclimate, the speed of the treadmill can then be accelerated slowly to 2m/min for 1-2 minutes and then 3m/min etc. If at any speed the mouse appears fatigued, refuses to run and/or is injured they must be removed from the study and the PI and facility manager must be notified immediately.
* Initial acclimation for 15 minutes a day for 2-3 days should be carried out before the initiation of the actual exercise regimen.

**2. Exercise Regimen:**

*At this point, the mice are deemed proficient and the desired belt speed is selected and the electric stimulus is turned on:*

* For our protocol the desired speed is 30 m/min, adjust the time for total of 30 minutes including the warm up time.
* Electric stimulus is a 200 millisecond (ms) pulse of electric current adjustable from 0.34 to 1.60 milliamperes (mA). The pulse is presented 1, 2 or 3 cycles per second (Hertz, Hz) (maximum voltage of 1.6). Individual toggle switches associated with each lane on the treadmill controls the application of the electric stimulus to the shock grids. We recommend the pulse to be started
* gradually from starting at 1 cycle and the minimum of 0.4 mA; this can be increased for the desired results up to the maximum of 1.6 mA.
* If the animal did not respond to the electrical pulse on the grid for 5 second for 3 times (seems acceptable as we previously tried it), the bead curtain and gentle hand push, this can be an indication signs for animal exhaustion. In such case the animal should be removed from the treadmill and returned to its cage (follow the procedure explained below in the following, additionally please consult exhaustion section for broader understanding of the exhaustion).
* In event of animal exhaustion the shock grid will be turned off, the animal is returned to their home cage and allowed to rest for 2-5 minutes. If the mice are deemed rested by the operator, they may be returned to the treadmill if the their group is still running
* Operators must stay focused at this stage of the experiment as animals will refuse to run and slide to the back of the treadmill where they can slip below the belt resulting in injury or death. If such an event takes place, the operator must immediately stop the treadmill and assess the animal’s health; depending on the severity of the injury euthanization may be required. The PI and facility manager must be notified immediately.
* At the end of the exercise regimen the shock grid is turned off and the belt speed reduced every 2-3 minutes until it comes to a complete stop. All animals will be returned to their home cage and observed for any signs of injury, pain/and or distress.

**Signs of Exhaustion**:

*The following criteria must be observed to deem the animal exhausted:* *This part is used as additional criteria to understand and prepare operators for other signs of exhaustion that are not covered in the SOP and might show up later, however the major criteria for removal due to exhaustion is already mentioned as part of the exercise regimen section.*

* Greater than 5 consecutive seconds on the shock grid without attempting to reengage the treadmill
* Spending greater than 50% of its time on the shock grid the third time a mouse is willing to sustain 2 seconds or more of shocking rather than return to the treadmill. Each operator should use common sense and, in addition to the criteria listed here, define exhaustion in his or her own terms. Please note operators should be aware that there are some mice that simply cannot keep up with the majority of similar mice. These are fairly rare, and they should be removed from the experiment whenever possible.

C. **Cleaning and Maintenance**:

After every use the treadmill and all parts will be cleaned with a mild solution of detergent, 70% alcohol and water. Also, after every use the tray underneath the treadmill that collects feces and urine will be cleaned and sanitized.

**References**

* Hintze, T and E. Shesely. Is a Mouse Like Any Other Mouse? J Mol Cell Cardiol. 34: 1283-1286, 2002
* Vilquin JT, Brussee V, Asselin I, Kinoshita I, Gingras M, Tremblay JP. (1998). Evidence of mdx mouse skeletal muscle fragility in vivo by eccentric running exercise. Muscle Nerve 21:567-576.
* Wei C, Penumetcha M, Santanam N, Liu YG, Garelnabi M, Parthasarathy S. Exercise might favor reverse cholesterol transport and lipoprotein clearance: potential mechanism for its anti-atherosclerotic effects. Biochim Biophys Acta. 2005 May 25;1723(1-3):124-7.