*- Maximal Work Test*

At PN33, all mice were exposed to the treadmill for 15 min each day during a 5 day acclimation period.  Each mouse was acclimated according to the following protocol: Day 1: 0 degree grade, 0 m/min, 0mA shock. Day 2: 0 degree grade, 5 m/min, 0mA shock. Day 3: 10 degree grade, 5 m/min, 0mA shock. Day 4: 10 degree grade, 10 m/min, 0.4 mA shock. Day 5: 10 degree grade, 10m/min, 0.75 mA shock.  Following a day of rest, a maximal work test was performed on PN39. After a 10 min warm up at 10 m/min (0 degree grade, 0.75 mA shock), the grade was increased to 10 degrees and the speed increased by 5 m/min every 2 min until exhaustion.  Exhaustion was determined when the mouse was no longer able to keep up with the treadmill and remained on the shock grid for longer than 20 seconds.  Maximal work was determined using the following equation:

Joules (J) = 9.8\*Speed (m/min)\*grade (radians)\* time (min)\* weight (kg).

*- Cardiorespiratory Fitness and Exercise Capacity in Mice*

Cardiorespiratory fitness (CRF) is positively associated with short- and long-term reductions in risk of cardiovascular disease for men and women (Gupta et al. 2011). Typically, CRF is assessed through measurement of maximal oxygen consumption (VO2max) during a graded exercise test. According to Fick’s Equation, VO2max is a product of cardiac output (Q) and Arteriovenous oxygen difference (A-vO2 diff); this considers both delivery of oxygenated blood and uptake of oxygen (Fick 1870). Cardiac output increases (through increases in heart rate and/or stroke volume) with exercise intensity in order to circulate oxygen and match the ATP (energy) demands of contracting skeletal muscle (Nystoriak et al. 2018).

In order to utilize appropriate methods to test our hypothesis, it is crucial to understand the strength and limitations in assessing CRF in mice. Assessment of human CRF involves incremental increases in exercise intensity (typically on a treadmill) with continuous measurement of oxygen consumption to establish VO2max. In mice, this can be measured using an enclosed metabolic treadmill; however, research published by Knab et. al*.* showed that measures of VO2max on sealed metabolic treadmills are not repeatable within the same mouse (Knab et al. 2009). This is due to the discontinuity between the mouse’s ventilatory stream and the gas sampling sensor, stress responses to shock administration, inability to use tail tapping for motivation, or due to rodent refusal to participate in the test (Knab et al. 2009).

Through the use of deconvolution equations or direct sampling from the mouse, the moment-to-moment values of VO2 can be extrapolated(Ferguson et al. 2019; Knab et al. 2009); however, these methods retain the issue of high equipment costs and difficulty of operation(Fernando et al. 1993). Through comparison with these equation-derived metabolic chamber VO2­max measurements, Fernando and colleagues were able to confirm that body weight and running speed could be used to predict VO2 with a low prediction error (2.4 ± 2.9%)(Fernando et al. 1993). Both body weight and running speed are used in the calculation of work:

*Work (Joules) = 9.8 \* maximal speed (m·min-1) \* grade (radians) \* time (min) \* weight (Kg)*

Combined with an incremental treadmill test to failure, this calculation can be utilized as a reliable means of estimating VO2 max. This logic has been repeatedly applied in the literature(Barbato et al. 1998; Ferguson et al. 2019; Knab et al. 2009; Lightfoot et al. 2001; Massett et al. 2005; Oydanich et al. 2019; Platt et al. 2015), with repeatable within-mouse work results being observed by Knab and colleagues(Knab et al. 2009). Thus, the use of maximal work equations will allow for determination of mouse CRF and exercise capacity as influenced by early life undernutrition.