Standard Operating Procedure for Data Collection and Sample Harvesting

This is a protocol for exercise training studies in terms of data collection and sample harvesting. Each study is unique, which could deviate from this protocol.

Before training

1. **CLAMS.** We put mice in metabolic cages for 5 full days which allows for simultaneous measurements of oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio (RER), food consumption, locomotor activity levels for 5 days. We could perform this at 7 weeks of age (or a week before training in your designed studies).

During training

1. **Body weight**. Measure and record body weight before training and every week on the 7th day of each of the training week.
2. **Running or weightlifting activity**. Download and quantify running or weightlifting activity and input the data as soon as you can so if there is any problem with running or weightlifting, you will have a chance to correct.

After training

1. **Body weight and body composition**. Measure body weight and body composition by EchoMRI after 4 weeks of training. This duration could be different depending on the experimental design.
2. **Behavioral tests**. Perform Open Field, Novel Object Recognition, Contextual and Cued Fear Conditioning tests in week 5 (or in the week after the designed training) while exercise training continues.
3. **GTT.** This test will be done after 3 days of animal handling and after 6 hours of fasting. Mice continue to exercise.
4. **ITT.** This test will be done after one day break and after 6 hours of fasting. Mice continue to exercise.
5. **VO2max test.** VO2max test will be done after 3 days of climatization (this could start on the day of ITT) and at ~9-10 am after overnight with no exercise. Animal will resume exercise.
6. **Endurance test.** Endurance test will be done 48 hours after VO2max test at ~9-10 am after overnight with no exercise. Animal will resume exercise.
7. **Echo Cardiography.** Echo cardiography with dobutamine stress will be done 48 hours after endurance test at 9-10 am after overnight with no exercise.

Sample harvesting

1. **Serum.** Measure body weight, tail vein blook glucose and collect blood sample (for serum) by cardiac puncture after overnight fasting.
2. **Brain.** mRNA, OCT (be sure of orientation for embedding) and protein for brain stem and hippocampus
3. **Heart.** Weight, mRNA, protein
4. **Liver.** mRNA, OCT, protein
5. **Kidney.** mRNA, protein
6. **Muscle.** Muscle weight for SO, PL and GA. PL for mRNA, OCT (cross-section), protein. GA freezing
7. **Fat.** iWAT and eWAT for mRNA, protein and OCT. For some metabolic studies, we may need to collect BAT.

	Sunday	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
Week 0		Weights CLAMS 1	CLAMS 2	CLAMS 3	CLAMS 4	CLAMS 5	Exercise -1 Lock Wheel
Week 1	Exercise 0 Lock Wheel	Weights 1 Unlock Wheel Start Exercise	Exercise	Exercise	Exercise	Exercise	Exercise
Week 2	Exercise	Weights 2 Exercise	Exercise	Exercise	Exercise	Exercise	Exercise
Week 3	Exercise	Weights 3 Exercise	Exercise	Exercise	Exercise	Exercise	Exercise
Week 4	Exercise	Weights 4 Exercise	Exercise	Exercise	Exercise	Exercise	Exercise
Week 5	Exercise	Weights 5 EchoMRI	Behavioural Tests	Behavioural Tests	Behavioural Tests	Behavioural Tests Handle mice	Handle mice
Week 6	Handle mice	GTT 6 Hr Fast	ITT 6 Hr Fast Acclimate 1	Acclimate 2	Acclimate 3 Lock Wheel Overnight	VO2 Max Resume Exercise	Exercise Lock Wheel Overnight
Week 7	Exercise	ETT	Exercise Lock Wheel Overnight	Echo	Fast/ Lock Wheel Overnight	Sample harvesting	