UNIVERSITY OF CALIFORNIA
RIVERSIDE

# Experimental Evolution of Exercise Physiology in House Mice Selectively Bred for High Locomotor Activity

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Biology

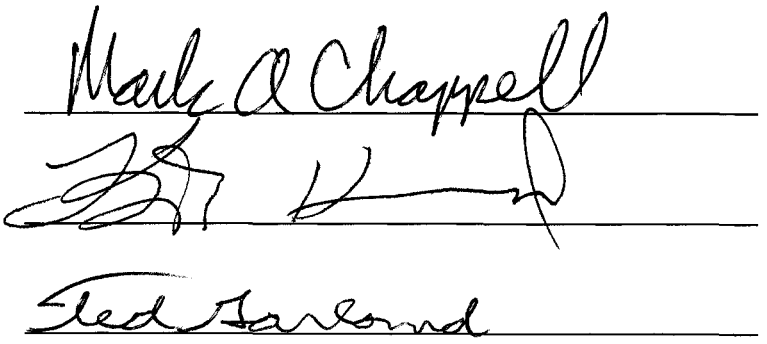
by

Enrico Landaeta Rezende

August 2005

Dissertation Committee:
Dr. Theodore Garland Jr., Chairperson
Dr. Mark. A. Chappell
Dr. Kimberly A. Hammond

The Dissertation of Enrico Landaeta Rezende is approved:



Committee Chairperson

University of California, Riverside

# APPENDICES

## APPENDIX 1 – METHODOLOGICAL DIFFERENCES BETWEEN MALES AND FEMALES $VO_2$ MEASUREMENTS ON WHEELS

(Applicable to Chapters 2, 3 and 6)

1) ***Individuals used and housing conditions*:** Males were retired breeders (generation 33 post-selection), and their housing conditions were not standardized before $VO_2$ measurements on the wheels, nor before $VO_2$max trials on treadmill. Females (generation 35 post-selection) were kept 4 per cage (2 S and 2 C per cage) right after weaning.

2) ***$VO_2$ trials on wheels, and acclimation period on wheels prior to measurements*:**

**Obs**: $CO_2$ analyzer was not calibrated everyday for wheel trials in males, data was not employed for the dissertation.

2.1) Males had access to wheels during 5 days before measurement in chamber, whereas females had access to wheels for 4 days before measurements (hence, males were actually measured on day 6, and females were measured during days 5 and 6).

2.2) Measurement on wheels lasted only 24 hours for males, whereas on females measurements lasted 48 hours. The 2500 initial samples in the first day of trial were not always used in analyses because chamber did not attain a steady-state and mice ran abnormally more probably because of the novel environment.

2.3) Measurements were performed simultaneously in two metabolic chambers for the females, whereas only one metabolic chamber was available for the initial trials in males.

2.4) Wheel resistance was estimated before and after each trial only for the females. No resistance was measured for males.

**3) *$VO_2$max on treadmill*:**

**Obs**: $CO_2$ analyzer was not calibrated everyday for treadmill trials in males, data was not employed for the dissertation.

3.1) Males were measured on the treadmill twice in consecutive days, separated in 4 batches between 28 Oct. 2002 and 20 Nov. 2002. The time-lag between measurements of $VO_2$ on wheels and on treadmill was not standardized. Females were measured with a standardized schedule (Table 2.1).

3.2) Males ran on a treadmill with a 15° slope, except for the first batch, that ran in a treadmill with no slope. Females ran on a 25° slope, which has been described to maximize the workload of treadmill running in mice (see Chapter 2).

**4) *$VO_2$max on Heliox*:** No measurement of $VO_2$max on heliox was performed in males. Females were measured in heliox two days after $VO_2$ wheel trials, following treadmill trials (Table 2.1).

## APPENDIX 2 – STEP-BY-STEP ANALYSES OF WARTHOG FILES

Corrections performed on Warthog files to obtain 1) estimates of $VO_2$, $VCO_2$ and wheel speeds from measurements of voluntary activity on wheels (each file contained 24 hours of measurement per individual); 2) estimates of wheel resistance measured before and after each trial, and 3) estimates of $VO_2$ and $VCO_2$ during measurements of forced exercise on treadmill and during cold-exposure in a Heliox atmosphere.

**Obs:** Measurements in Heliox were performed only in Females (see Appendix 1).

### 1) *$VO_2$, $VCO_2$ and wheel speed*

1.1) Synchronization of $O_2$ and $CO_2$ channels.

Lag-time correction (ch. 1 = 18 sec, ch. 8 = 15 sec).

1.2) Synchronization of $O_2$ and $CO_2$ with wheel-speed channel

This was performed according to the test file "**lag time test 1/29/03**".

Lag-time correction (ch. 1 and 2 = 20 sec, ch. 8 and 9 = 15 sec).

1.3) Baseline correction in wheel speed.

Channels 4 and 12 duplicated before baseline correction (new channels are 13 and 14).

**Obs:** Peaks in wheel speed removed in duplicate channels from animals 37284 and 37431, no interpolation markers added.

1.4) Wheel speed absolute values.

Channels 13 and 14 duplicated (new channels 15 and 16).

1.5) Baseline correction in $O_2$ and $CO_2$.

Channels 1 and 2, 8 and 9 were duplicated before correction (new channels are 17 and 18, 19 and 20).

1.6) Visual inspection of $O_2$ and $CO_2$ channels.

Weird peaks removed (probably changes in voltage). The criterion for peak removal: if same peak was observed in the two different wheels (normally this occurred between $O_2$ analyzers). No interpolation markers were included.

**Obs:** Backup channels of $O_2$ and $CO_2$, corrected up to this level, were duplicated (Channels 21 to 24).

1.7) Reference removal in $O_2$ and $CO_2$ channels.

Data below 0.01 and additional 25 cases in each side were removed. Interpolated data was used, and markers regarding interpolated data were added according to interpolation in channel 17 ($O_2$ wheel 1; references were removed in channels 17 to 20).

1.8) Smoothing data in $O_2$ and $CO_2$ channel.

Smoothing of 7 by 20 (7 data points averaged in 20 cycles), in channels 17, 18, 19 and 20.

1.9) Instantaneous corrections (IC) in $O_2$ and $CO_2$ channels.

Corrections performed in channels 17 to 20. Effective volume was 17000 ml, $CO_2$ not absorbed (mode 2 in Warthog $O_2$ IC and mode 1 in $CO_2$ IC).

**2) *Resistance files***

2.1) Baseline correction

Channels 1 and 2 were duplicated before baseline corretion.

**3) *$VO_2$ and $VCO_2$ on treadmill***

3.1) Visual inspection of all channels (4 in total)

Electrical spikes removed in $O_2$ data (all other channels checked as well).

3.2) Synchronizing $VO_2$ and $CO_2$ channels

$VO_2$ channel was 6 seconds behind $CO_2$ channel (these channels were not synchronized with belt speed channel, although there was almost no lagtime in $CO_2$ [just a couple of seconds, considering the switch between reference and the chamber]).

3.3) Baseline correction $VO_2$ and $CO_2$

Both $VO_2$ and $CO_2$ channels were duplicated (new channels 5 and 6).

3.4) Smoothing the data

Channels 5 and 6, smoothing of 3 by 3.

3.5) Instantaneous correction (IC) in $VO_2$ and $O_2$ channels

Corrections performed in channels 5 and 6. Effective volume was 903 ml, $CO_2$ not absorbed before chamber (modes 2 in Warthog for $O_2$ IC and 1 in $CO_2$ IC).

**4) *$VO_2$ and $VCO_2$ in Heliox***

4.1) Visual inspection of all channels (4 in total)

4.2) Baseline correction $VO_2$ and $CO_2$

4.3) Synchronizing $VO_2$ and $CO_2$ channels

$VO_2$ channel was 6 seconds behind $CO_2$ channel.

4.4) Smoothing data $VO_2$ and $CO_2$

Both $VO_2$ and $CO_2$ channels were duplicated (new channels 5 and 6).

Channels 5 and 6, smoothing of 3 by 3.

4.5) $VO_2$ and $CO_2$ transformation

Steady-state condition, so instantaneous correction was not applied.

$CO_2$ not absorbed (modes 2 in $VO_2$ and 1 in $CO_2$).