Mitochondrial respirometry reference values from permeabilized mouse soleus muscle fibers.

MitoEAGLE – WG2 pilot study.
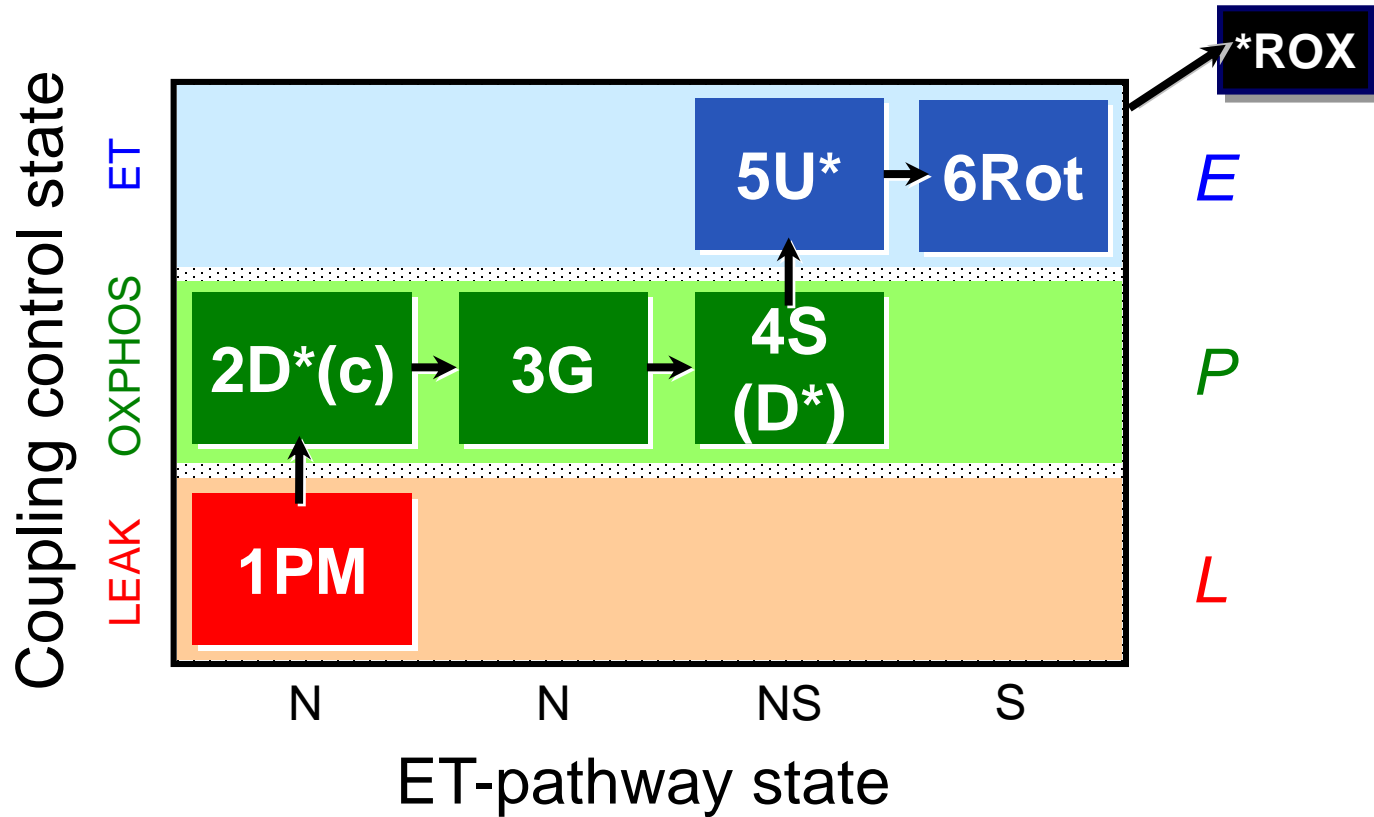
Garcia-Roves PM, Chabi B, Cortade F, Doerrier C, Dubouchaud H, Gama-Perez P, Grefte S, Irving B, Ost M, Pesta D.
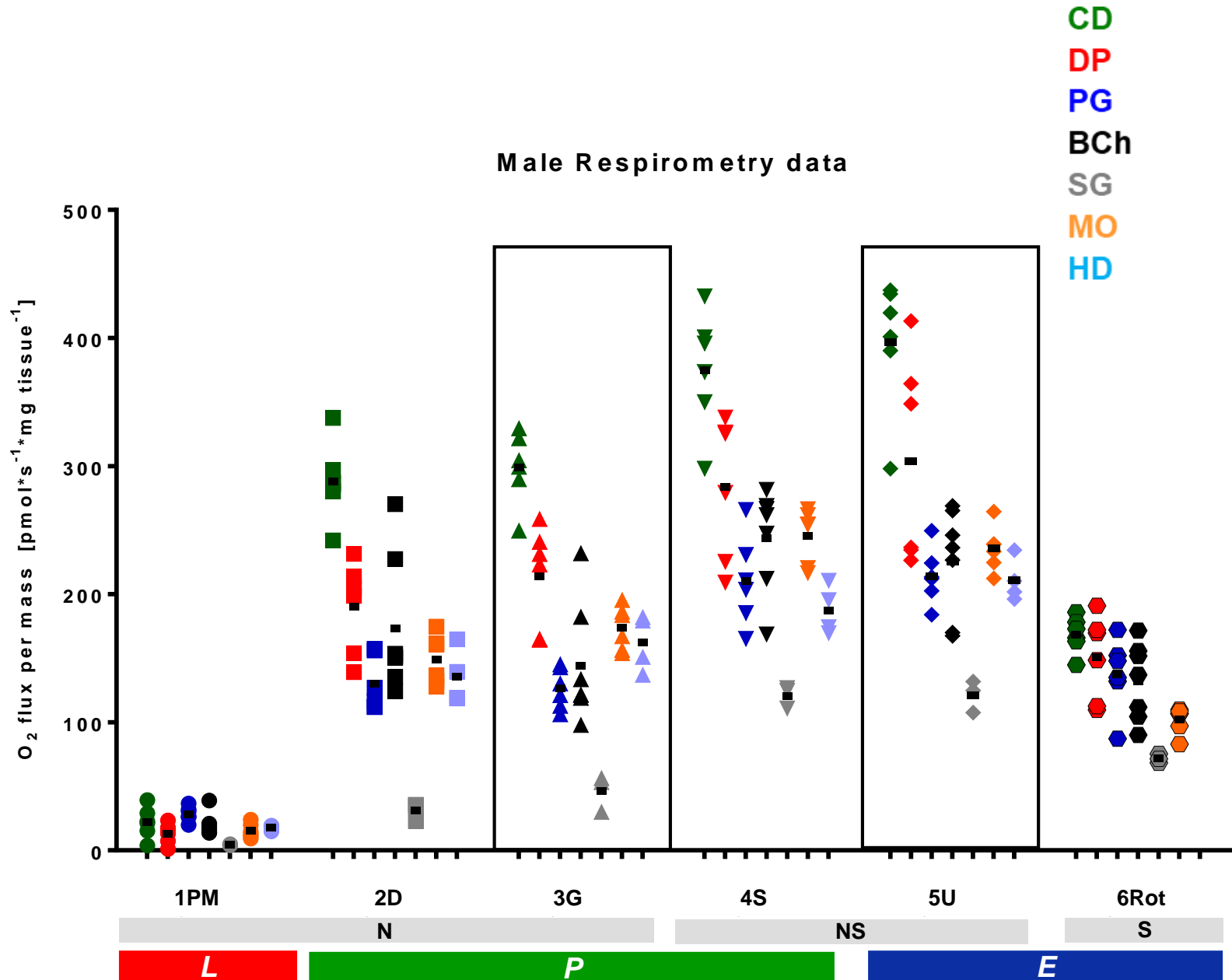
Mouse model

- Mouse strain: C57BL6 J

- Gender: male (N=4) and female (N=4), total N=8

- Age: 14-20 weeks (mouse);

- Skeletal muscle type: soleus

PROPOSAL – EXPERIMENTAL DESIGN: PROTOCOL
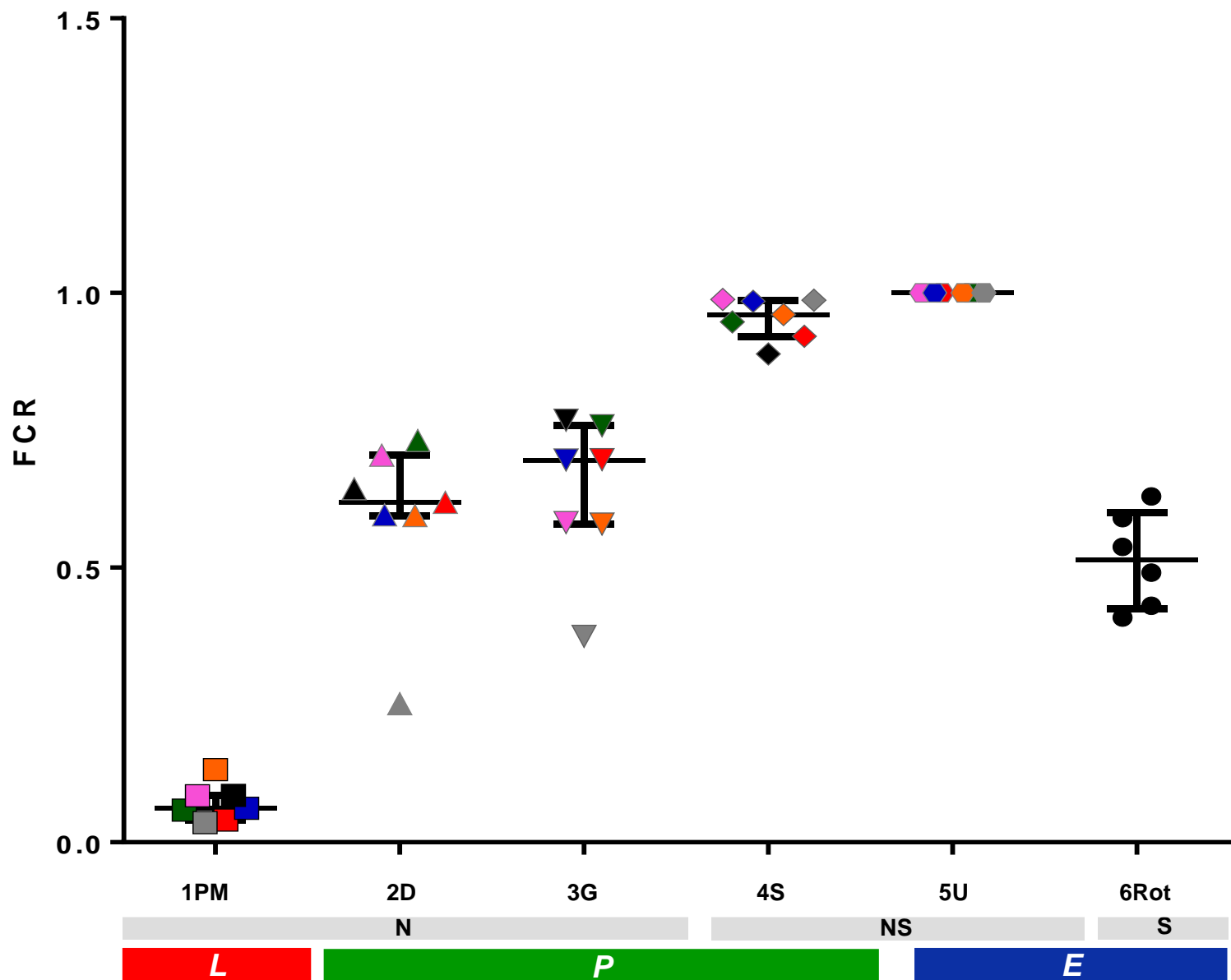
ANESTHESIA

- **No anesthesia. If it is required the use of anesthesia → report it**

SAMPLE PREPARATION

- Preservation media: BIOPS

- Mt-preparations: permeabilized fiber (pfi)

- Mechanical separation of fibers: all soleus in mouse

- **Saponin**: 50 µg/ml f.c. during 30 minutes

- Wash step: MiR05-kit

- Weight of the fiber bundle: blot for 40 s in blotting paper and then weight 1.0 - 1.4 mg w/w of tissue per chamber.

MITOCHONDRIAL RESPIRATION

- Any instrument.

- Respiration media: MiR05-kit

- Temperature: 37 °C

- **Oxygen regime**: High oxygen (400-250 µM)

- Reoxygenations: pure oxygen (optimal max: 400 µM)

PROPOSAL – SUIT PROTOCOL

1PM;2D;2c;3G;4S;4D;5U;6Rot;7Ama;8TmAs;9Azd
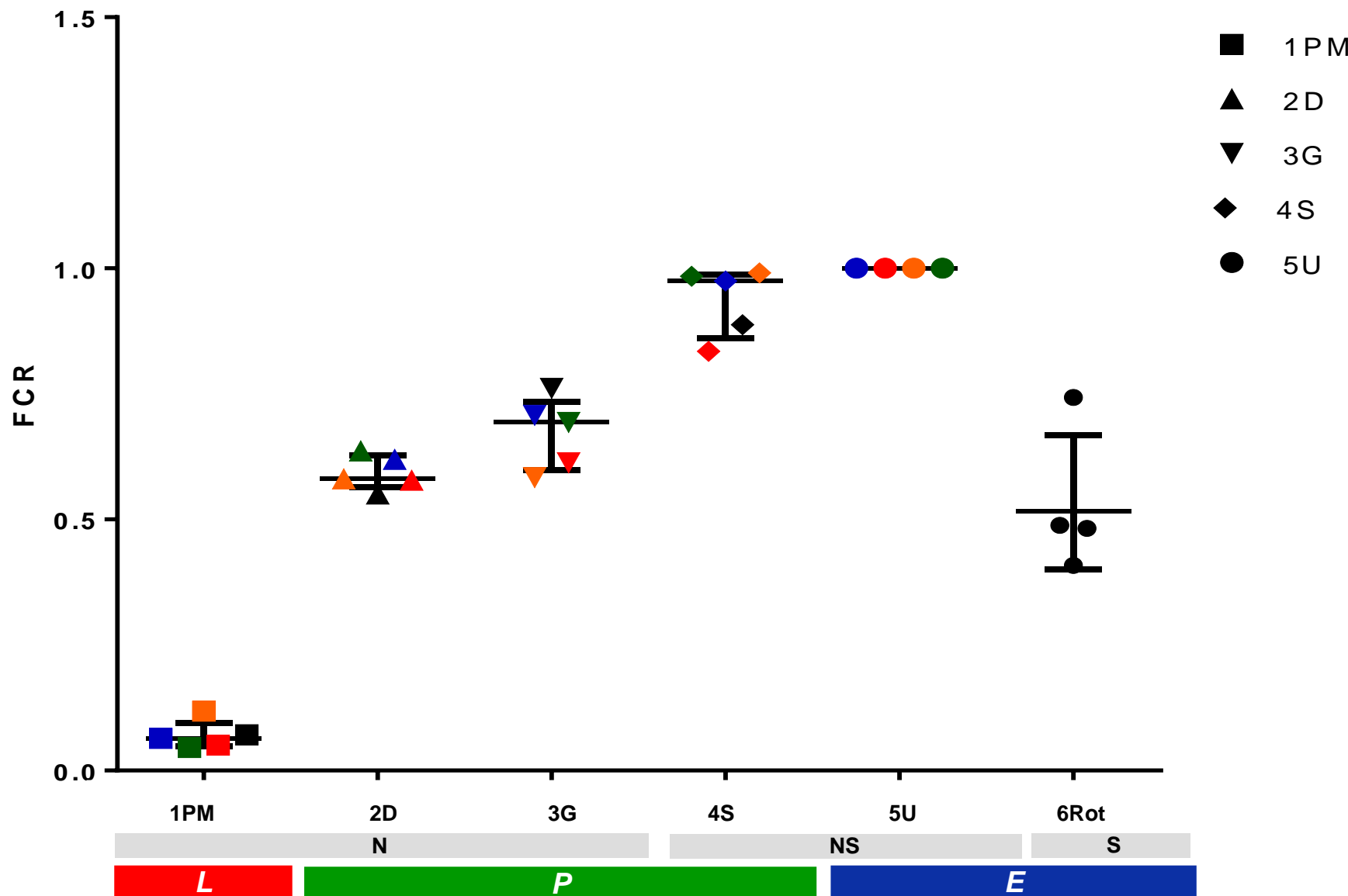
MALE

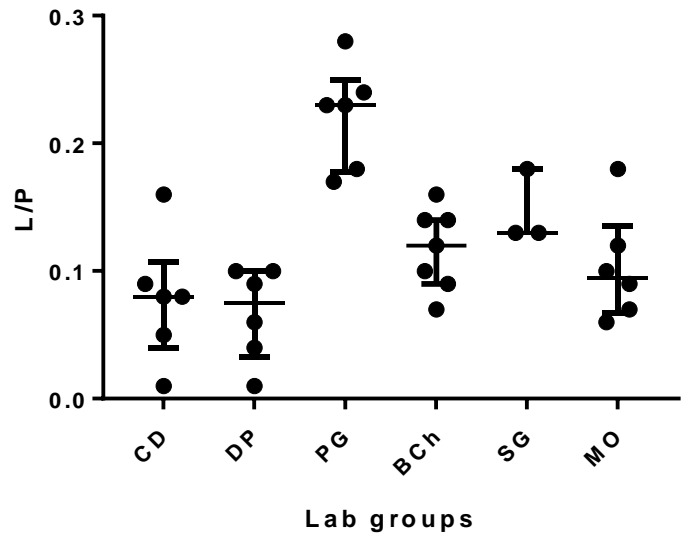Male Respirometry data

Male FCR median
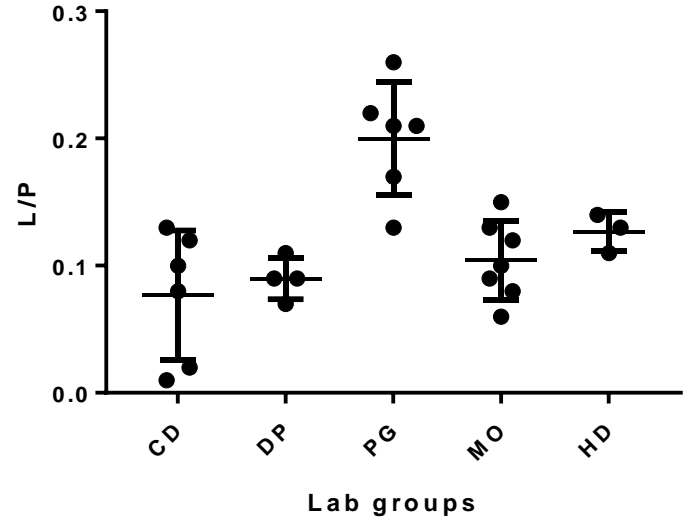
Female Respirometry data

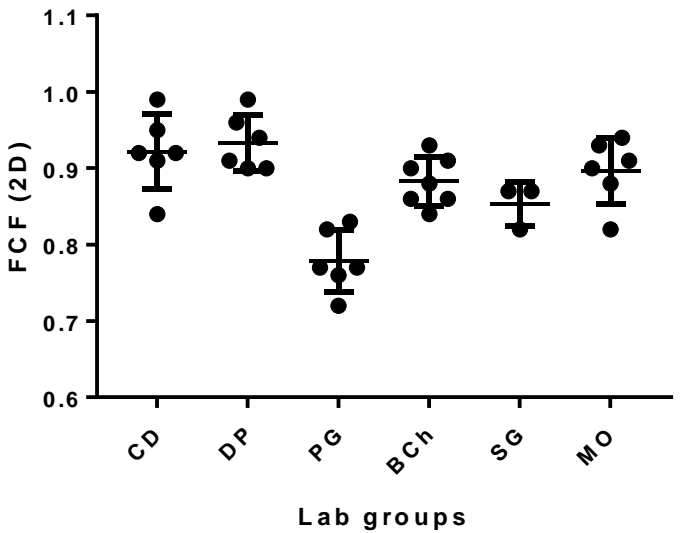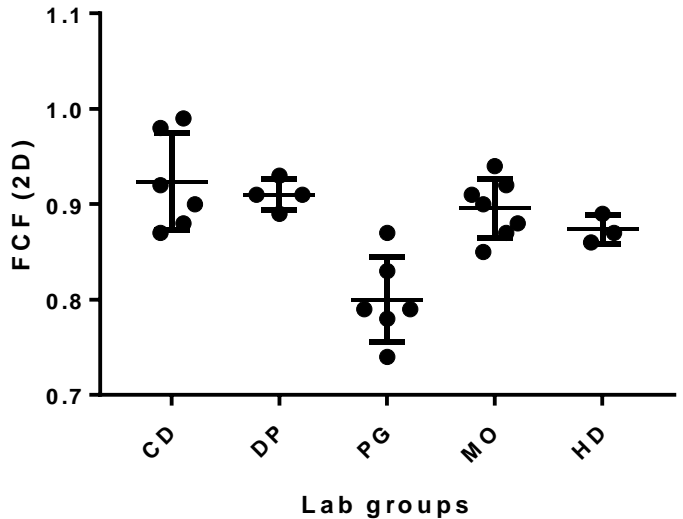Female FCR median

Flux control factors

Male L/P

Female L/P

Male FCF(2D)

Female FCF(2D)

Future actions

Potential aspect to improve to reduce variability:

- Reoxygenation of the chamber
- Purity of preparations (connective tissue contamination)
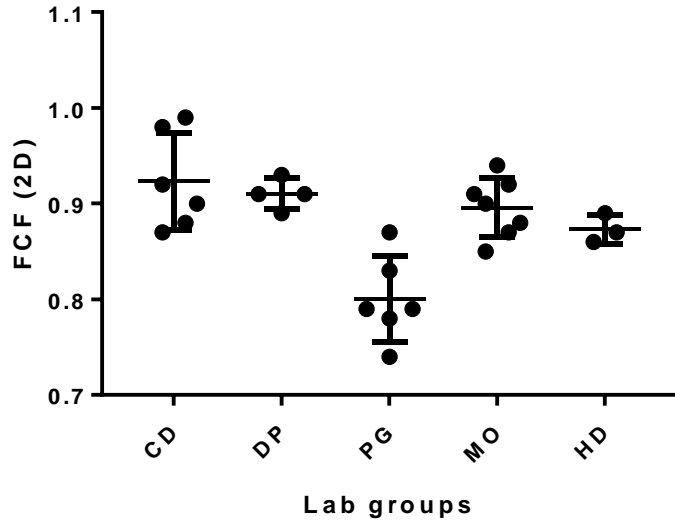- Muscle wet weight (environmental humidity)
- Chemicals
- Others

Get a consensus and invite all labs interested on taking part of this study
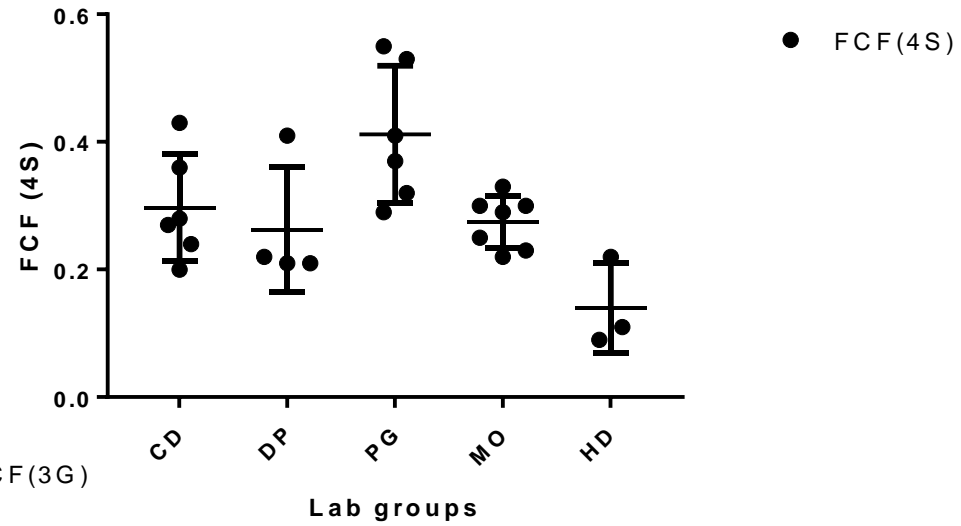
- Set a deadline for data collection
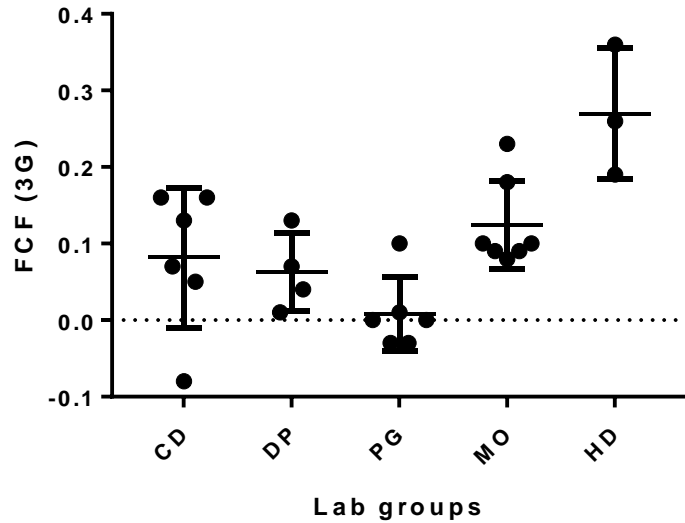
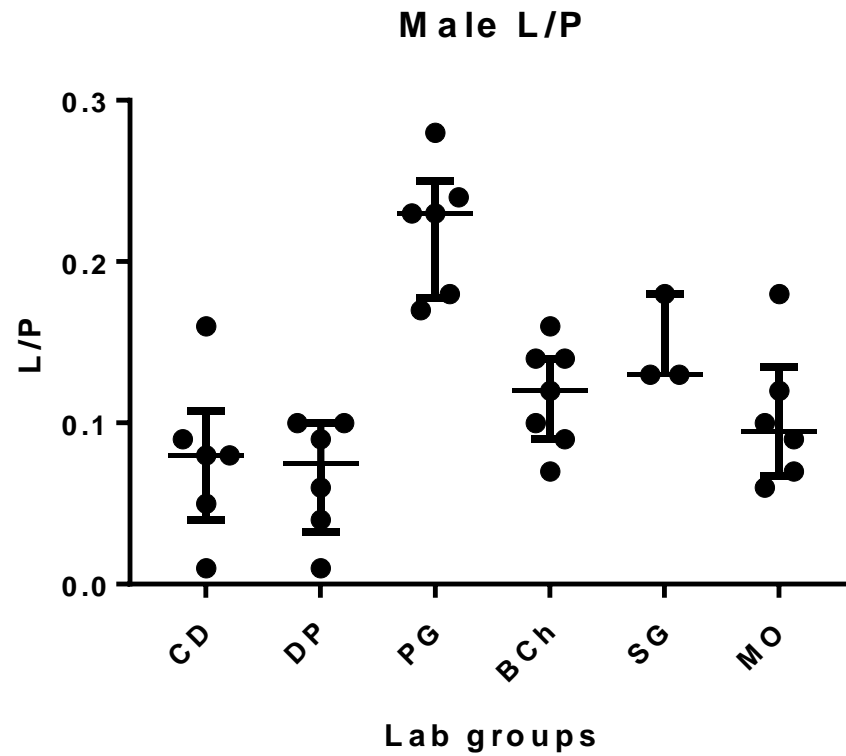**Data analysis and manuscript preparation**

FEMALE

Male L/P
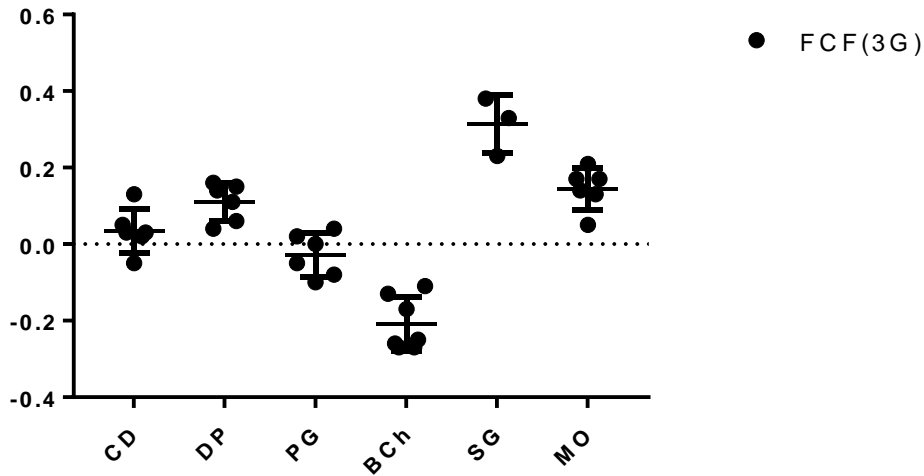
Male FCF(2D)

Male FCF(4S)

Male FCF(3G)