



WG2 SUMMARY

MITOEAGLE 2017, Obergurgl AT

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Implementation of a reference protocol as a tool for instrumental and technical quality control in muscle tissues.

Species:

- Human
- Mouse
- Rat
- Other species (horses, dogs, cows, fish, insects...)

Muscle tissues:

- Sk muscle.
- **Heart**

Mt-preparations:

- Permeabilized fibers (pfi)
- **Isolated mitochondria (imt)**
- **Tissue homogenate (thom)**

Mouse and rat model

- Mouse strain: C57BL6 (N or J, suggested J)
- Rat strain: Wistar
- Gender: male (N=4) and female (N=4), total N=8
- Age: 14-20 weeks (mouse); 200-300 g (rat)
- Skeletal muscle type: soleus

ANESTHESIA

- **Anesthesia?**

SAMPLE PREPARATION

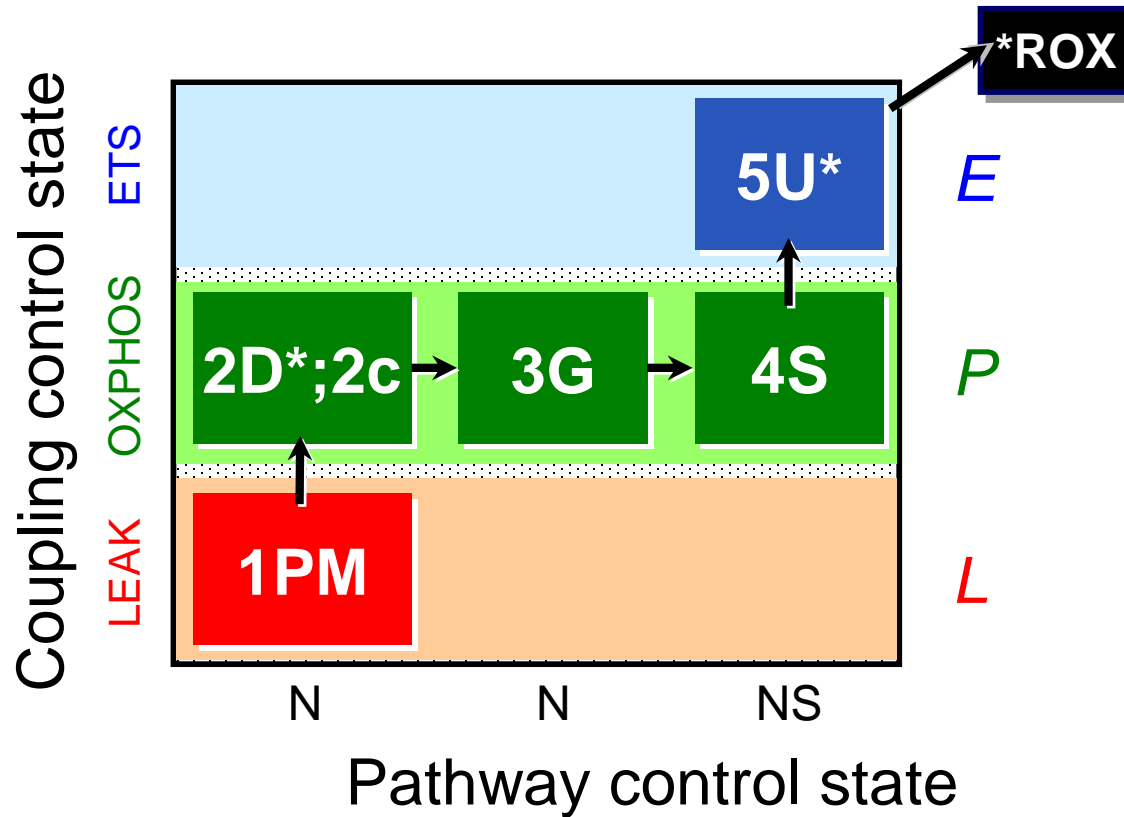
- Preservation media: BIOPS
- Mt-preparations: permeabilized fiber (pfi)
- Mechanical separation of fibers: all soleus in mouse
- **Saponin**: time and concentration to be determined!
- Wash step: MiR05-kit
- Weight of the fiber bundle: blot for 40 s in blotting paper and then weight **1-1.4** mg w/w of tissue per chamber.

MITOCHONDRIAL RESPIRATION

- Any instrument.
- Respiration media: MiR05-kit
- Temperature: 37 °C
- **Oxygen regime**: High oxygen (400-250 μM)? Other oxygen range?
- Reoxygenations: pure oxygen (optimal max: 400 μM)

PROPOSAL – SUIT PROTOCOL

1PM;2D;2c;3G;4S;5U;6Ama



- DatLab 7. DatLab protocol.

Human

- Skeletal muscle type: *vastus lateralis*
- Gender: male and female
- Age: young, middle age and old
- Several ethnicities
- BMI and physical activity
- Epinefrin free
- Details to be discussed:
 - Medication
 - Biopsy technique

PROPOSAL – EXPERIMENTAL DESIGN: PROTOCOL



- Time of collection (BIOPS)
- SUIT protocol: to be defined by the Human-WG2

NEXT STEPS



- Pilot study (saponin concentration and time exposition).
- Contact with laboratories interested.



SUMMARY SESSION

AFTER DISCUSSION (SUMMARY SESSION)



- Rotenone (Rot) was excluded to the SUIT protocol, mainly because in *soleus* it takes longer to inhibit CI (~15 min). However, it has to be added to the SUIT protocol because it is important for the evaluation of the quality of the preparation. S-pathway in the ETS state is more stable and it can provide information about a decrease in NADH-linked respiration related to the mitochondrial preparation.
- Is there another CI inhibitor that we could use? Maybe REGM inhibitor? Maybe is not commercially available?
- CIV analysis is optional: it will be added in some groups (which will provide the reference values). It will be optional for evaluating the quality of the preparation in a specific laboratory.
- Respect to succinate (S), 50 mM (f.c.) of S will be used in the SUIT protocol.
- Some groups of the WG2 have to start a pilot study to check everything before the official call is generated.

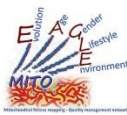
AFTER DISCUSSION (SUMMARY SESSION)



- Saponin conditions (time and concentration) can be evaluated for soleus in the pilot experiment.
- Method for the sacrifice of the mouse have to be defined. Could be applied theb CLP-method in all the countries involved?
- In the study for isolated mitochondria, data and information from Charles Hoppel results can be used as a reference.

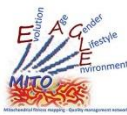
- Next steps:
 - Pilot study (some groups from WG2).
 - Analysis and conclusions for the pilot study (ideal deadline: Hradec Kralove CZ, 15-17 Nov 2017).
 - Call for the collection of data (experiments) for the MITOEAGLE data repository on muscle tissues (ideal deadline: beginning of 2018).
 - Publication n°1 with the methods and the reference values: 2018.
 - Publication n°2 showing the proper SUIT protocols for the evaluation of specific defects (CI-related, CIII, FAO, OXPHOS ...). Conceptually input from C.Hoppel and E.Gnaiger.
 - In human, the SUIT protocols have to be optimized.

AFTER DISCUSSION (SUMMARY SESSION)



- Next steps:
 - In human, and perhaps in another animal models (mouse, rat), if new experiments have to be performed: two different SUIT protocols could be applied. The second SUIT protocol should evaluate FAO (which FA? C. Hoppel suggestion: Pal and then Oct?). Perhaps target RP2 (<http://www.bioblast.at/index.php/1OctM;2D;3P;4S;5U;6Rot->).
 - High malate concentration (2 mM f.c.) is not a problem in human sk.muscle (no anaplerotic pathways).
 - Maybe two different SUIT protocols can be done in sk.muscle (mouse). The reference protocol (defined in the slide n°14 and FAO evaluation: target RP2).

SUIT PROTOCOL – sk. Muscle (soleus), mouse

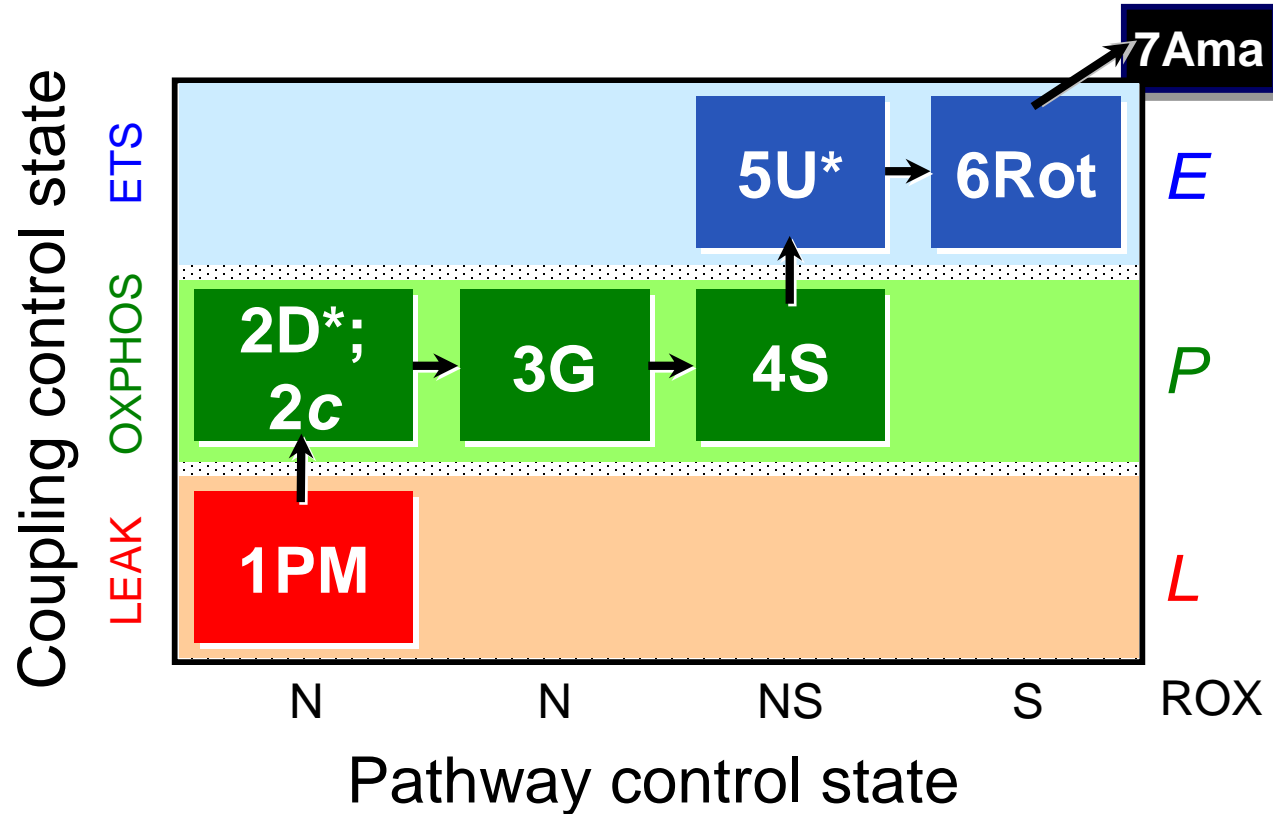


1. To do: 1PM;2D;2c;3G;4S;5U;6Rot;7Ama

2. Optionally, do SUIT protocol 1 including CIV assay:

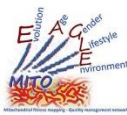
1PM;2D;2c;3G;4S;5U;6Rot;7Ama;8AsTm;9Azd

SUIT n°1



- DatLab 7. DatLab protocol.

SUIT PROTOCOL – sk. Muscle (soleus), mouse



1. To do: 1PM;2D;2c;3G;4S;5U;6Rot;7Ama

2. Optionally, do SUIT protocol 1 including CIV assay:

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

SUIT n°2

