Obergurgl, 2017 July 28-29

Subject | Minutes of the MITOEAGLE Working Groups Workshop of COST Action CA15203 in Obergurgl: *"Mitochondrial mapping: Evolution - Age - Gender - Lifestyle - Environment"*

Minutes prepared by Zuzana Sumbalova

I. MITOEAGLE Working Groups Workshop: 2017 July 28

1. Welcome to participants

The participants were welcomed by Erich Gnaiger, the goals of the workshop and the programme was outlined, participants presented their interest in joining specific WG and specific topics

2. Participants

see Annex 1 – Attendance list

- Number of participants: 42
- COST participants 36 (day 1: 36 - day 2: 36)
- total signed 42

3. Session F1 **Selected presentations WG2, WG4** *(Chair: Z. Sumbalova)*

Luciana Ferreira: Doxorubicin toxicity and mitochondrial dysfunction in mouse induced pluripotent stem cells-derived cardiomyocytes.

Luiz Crisostomo: Glycerol metabolism in testicular cells – a mitochondrial tale that may control male reproductive potential.

Guida Bento: Isolation of human urine-derived stem cells for mitochondrial function profile assessment.

Roberto Scatena: Cancer stem cell differentiation induced by PPAR ligands. Which role for an inhibition of mitochondrial respiratory chain? *(poster)*

4. Session F2 **Selected presentations WG1, WG2** *(Chair: L. Garcia-Souza)*

Pablo Garcia-Roves: MITOEAGLE data repository on muscle tissues - current status.

Carolina Doerrier, Giovanna Distefano: Upper limit of OXPHOS capacity: effect of oxygen and ADP. The presentation of results from joint work on skeletal muscle fibres – comparison of respiration with MiR06 vs Buffer Z.

Andras Meszaros: Personal proficiency and reproducibility of experimental results: a comparative study on respirometric analysis of HEK cells.

5. Session F3 **Selected presentations WG2** *(Chair: P. Garcia-Roves)*

Jozef Ukropec: Could functional state of muscle mitochondria reflect exercise-induced changes in insulin sensitivity, glucose tolerance and cognitive performance in individuals with mild cognitive impairment?

Kasja Pavlovic: Establishing cellular model for studying mechanisms of muscle insulin resistance: towards understanding the role of mitochondria.

Adela Krajcova: Developing a method of assessing mitochondrial functions in homogenates of human heart muscle.

Beatrice Chabi: Optimization of permeabilized fibers preparation for mitochondrial respiration measurements using Design of Experiments methodology. *(poster)*

Timea Komlodi: Common pitfalls in the everyday work with respirometry.

6. Session F4 **WG workshops** (Participants attended WG discussion/working tables)

WG1- terminology
WG2 - muscle
WG3 - liver
WG4 - cell lines
WG4 - blood cells

II. MITOEAGLE Working Groups Workshop: 2017 July 29

7. Session G1 **Oxidative and nitrosative stress** *(Chair: L. Chrisostomo)*

Javier Iglesias-Gonzalez: Oscillations in mitochondrial ROS production as a new mechanism for cellcycle control.

Timur Gainutdinov: Different ways of reactive oxygen species (ROS) formation by brain mitochondria.

Herve Dubouchaud: Effects of a single bout of exercise on the production of reactive oxygen species in muscle and liver isolated mitochondria.

Jordi Muntane: Integration of oxidative and nitrosative stress in the overall cell death signaling induced by Sorafenib in hepatoma cells.

8. Session G2 **Presentations WG4: blood cells** *(Chair: A. Molina)*

Luiz Garcia-Souza: Towards a database on PBMC mitochondrial respiration: first steps on a literature survey linked to the MitoFit experimental data.

Beata Velika: High resolution respirometry and viability of cryopreserved blood cells.

Zuzana Sumbalova: Comparison of methods for PBMC isolation: the effect on yield, purity and respiration.

Brian Irving PBMC and T-cell Mitochondrial Function in Humans: LSU Update

Karolina Siewiera Altered blood platelet activation is not related to elevated mitochondrial respiration in rat model of streptozotocin-induced diabetes - preliminary results

9. Session G3 **Discussion in WG** (Splitting according WG interests to separate WG)

WG4 - blood cells
WG4 - cell lines
WG3 - liver
WG2 - muscle
WG1 - terminology

- **Summary from WG discussions presented by selected WG participants** (see SUMMARY bellow)
- **Invitation by Pavla Stankova to the next MiP2017/MitoEAGLE event in Hradec Kralove CZ**, 15-18 Nov 2017. 12th Conference on Mitochondrial Physiology: The role of mitochondria in lifestyle and metabolic syndrome - COST MitoEAGLE perspectives.

  http://www.bioblast.at/index.php/MiP2017/MitoEAGLE_Hradec_Kralove_CZ

SUMMARY from WG discussions:

WG4 blood cells summary *(prepared by L. Garcia-Souza and Z. Sumbalova AT)*

Participants: Kimberly Amick, Anthony Molina, Beata Velika, Brian Irving, Jozef Ukropec, Karolina Siewiera, Lara Nogueras, Luiz Garcia, Zuzana Sumbalova

Points discussed/shared:

- Isolation protocols: Anticoagulants; Handling of samples; Media usage; Storage time
- SUIT protocols
- Overall experiments results
- Collaborations

Suggestions and decisions:

- There was no decision in unification of protocols yet.
- The isolation procedure and SUIT protocols used in Oroboros and presented in Verona are available on the website: http://www.mitoeagle.org/index.php/MiPNet21.17_BloodCellsIsolation
- The isolation protocols used in different labs should be shared by e-mails with complete information (decission from the meeting in Verona – the template for filling the information about the isolation and cryopreservation procedure will be available on the website: http://www.mitoeagle.org/index.php/Talk:WG4_MITOEAGLE_data:_blood_and_cultured_cells). The protocols presented in Barcelona and Obergurgl meetings could be shared by e-mails.
- The protocols for respiration with representative values should be collected to compare the effect of different isolation procedures on respiration and to start building the database. All protocols and data will be collected and by Elisa Calabria and Zuzana Sumbalova, summary will be given in next meetings.
- To assure comparability of data between research groups, using two respiration media are recommended: MiR06+10 mM pyruvate for intact cells and MiR06Cr for permeabilized cells.
- Experiment will be done by Brian Irving exploring the effect of blood storage (up to 24h) on respiration of PBMC

WG4 cell lines summary *(prepared by R. Porter, IE)*

Discussion: Considerations when measuring, and "comparing and contrasting" oxygen consumption in cultured cells (cultured primary cells, stem cells, other cell lines)

1. Is there a PRIMARY CELL REFERENCE? *e.g.* primary hepatocytes to compare with HepG2's.

2. PASSAGE NUMBER:

Does cellular mitochondrial oxygen consumption wane with increased passage number?

(a) primary cell cultures, *e.g.* maximum passage number 7/8 in sertoli cells

(b) Cell lines, relationship with passage number not too well studied

Eveline HUTTER, Kathrin RENNER, Gerald PFISTER, Petra STOCKL, Pidder JANSEN-DÜRR and Erich GNAIGER: Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. Biochem. J. (2004) 380, 919–928.

3. ASSESSING BASELINE PARAMETERS BEFORE ASSESSING CELLULAR OXYGEN CONSUMPTION

(a) Is division symmetrical?

(b) What is the doubling time?

(c) Assess cell-cycle profile.

(d) Markers of cell type/stem cell type (Q-RTPCR, FACS, enzymes)

(e) Karyotyping

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4. MEDIUM to be USED

(a) Medium used *e.g.* DMEM, RPMI etc

(b) Glucose concentration is regularly a major variable

(c) Are fatty acids in the medium/to be added to the medium? saturated/unsaturated

(d) Stem cells, using KSR with/instead for FBS?

5. STP v NORMOXIA v HYPOXIA

Consideration should be given to growth or at least treatment/analysis at conditions equivalent to those *in vivo e.g.* Normoxia to minimise ROS damage

6. ASSESSMENT of OXYGEN CONSUMPTION not due to OXIDATIVE PHOSPHORYLATION

Usually more in cell lines

(a) NOS

(b) NOX

(c) XO

(d) HO (Nrf2)

(e) MAO

(f) CytochromeP450 related enzymes

(g) Peroxisomal (catalase)

7. CULTURE TYPE

(a) 2D

(b) 3D matrix cancer constructs: perhaps diffusion limiting for direct oxygen consumption measurements but fluorescent markers of bioenergetics function could be used to get a in vitro bioenergetic profile of a solid tumour

8. QUESTIONS at the round table discussion yesterday

(i) Cell permeabilization was the main focus

ACTIONS

9. Review article(s) acting as a database and reference article comparing and contrasting bioenergetics studies

The review(s) should contain

(i) cancer cell lines (taking into consideration source type, differentiation state, phenotypic state *etc*)

(ii) Stem cells (IPS, adult stem cells, embryonic)

(iii) Primary cell cultures

(iv) Reference to freshly isolated primary cell data where possible

(v) Attempts to compare published data is a standardised comparable format

(vi) Recommendation for a standard terminology to be used when working with cultured cells (from WG1)

10. Division of labour for cultured cells for which there may be significant oxygen consumption data:

Hepatocytes:- Jordi Muntané, Roberto Scatena other volunteers

Breast Cancers: Roberto Scatena, Richie Porter, other volunteers

Kidney cells: Andras Meszaros, other volunteers

Sertoli cells: Luís Crisostomo, Tiana Dias, Raquel Bernardino, other volunteers

Stem cells: Raj Rao, Shilpa Iyer, Guida Bento, other volunteers

Muscle cells *e.g.* C_2C_{12} cells Kasja Pavlovic, Erich Gnaiger, volunteers needed

Fibroblasts: Pidder Jansen-Dürr, Erich Gnaiger, other volunteers

Bone marrow derived macrophages: volunteers needed

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Lung: Porter, other volunteers needed

Other cells types: Cancer stem cells (Roberto Scatena), cardiomyoblasts (Luciana Ferreira)

WG3 summary *(prepared by O. Sobotka, CZ)*

Participants: Pavla Stankova, Jordi Muntane, Ondrej Sobotka and Pablo Garcia.

Discussion about possible interlaboratory cooperation, mainly focused on future exchange research internships. We also discussed how to proceed in next months of collecting respirometry data in liver group. All participants will receive an email in upcoming weeks with clear instructions and recommendations.

WG2 summary *(prepared by C. Doerrier, AT)*

Participants: Chabi B, Doerrier C, Distefano G, Dubouchaud H, Garcia-Roves PM, Irving BA, Isola R, Krajcova A

Aim: 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)**

PROPOSAL – EXPERIMENTAL DESIGN

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?

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

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- **Oxygen regime**: High oxygen (400-250 µM)? Other oxygen range?
- Reoxygenations: pure oxygen (optimal max: 400 µM)

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

PROPOSAL – DATA REPOSITORY

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

- 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.

AFTER DISCUSSION (SUMMARY SESSION)

- Rotenone (Rot) was excluded to the SUIT protocol, mainly because in soleus it takes longer to inhibite 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 commercialy 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 laboratiory.
- 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.
- 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 the cervical dislocation 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.

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- Publication n°2 showing the proper SUIT protocols for the evaluation of specific defects (CI-related, CIII, FAO, OXPHOS …). Conceptually imput from C.Hoppel and E.Gnaiger.
- In human, the SUIT protocols have to be optimized.
- In human, and perhaps in another animal models (mouse, rat), if new experiments have to been 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°15 and n°16 and FAO evaluation: target RP2).
- 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

WG1 summary

The terminology manuscript was revised during WG sessions and developed further, contributing co-authors were added. Erich Gnaiger will work on completing the manuscript, the COST members and other collaborators are asked to read the draft and send the feedback to E. Gnaiger until September 18th.

The up-to date version of the manuscript could be downloaded here:
http://www.bioblast.at/index.php/The_protonmotive_force_and_respiratory_control

The manuscript on 'Mitochondrial respiratory control' is in preparation as a position statement in the frame of COST Action CA15203 MitoEAGLE. Up to now, the list of co-authors is mainly based on previous MitoEAGLE Working Group Meetings. In the bottom-up spirit of COST, this is an open invitation to scientists and students to join as co-authors, to provide a balanced view on mitochondrial respiratory control, a fundamental introductory presentation of the concept of the protonmotive force, and a critical discussion on reporting mitochondrial respiration in terms of metabolic flows and fluxes. We plan a series of follow-up publications by the MitoEAGLE Terminology Group, to increase the scope of consensus-oriented recommendations and facilitate global communication and collaboration.

LIST OF ANNEXES AND LINKS

Annex 1 – Attendance List

Attendance List_signed2.pdf

Links:
Programme, abstracts, presentations and agenda:
http://www.bioblast.at/index.php/MiPschool_Obergurgl_2017#Programme_MITOEAGLE_Working_Groups_Workshop

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