

MitoEAGLE WG2 overview (Sept 2018)

- Current WG2 projects
 - **Human *vastus lateralis* pfi study** (MiR05, Buffer Z)
 - Data analysis
 - Workshop (discussing data, statistician, preparing manuscript)
 - **Reference values for skeletal muscle pfi** (mouse *soleus* study)
 - Additional information from participants. Collecting all data
 - Extended deadline for other participants willing to take part in this study
 - Data analysis
 - Workshop (discussing data, statistician, preparing manuscript)
 - **Metabolic signatures of human myotubes** (Review article)
 - Next steps
 - **Heart** (review)
 - Next steps
 - **Suggestions:**
 - **Skeletal muscle article - comparative biology**

Inter-laboratory study on human permeabilized myofibres: effects of fibre preparation, respiration buffer, and blebbistatin on mitochondrial respiratory capacity and oxygen limitation.

Doerrier C, Garcia-Roves PM, Distefano G, Pesta D, Gama-Perez P, Soendergaard SD, Chroeis KM, Gonzalez-Franquesa A, Coen P, Goodpaster BH, Larsen S, Gnaiger E

6 groups (Austria, Denmark, Germany, Spain, USA)



MiP2018/MitoEAGLE Jurmala LV

20.09.2018

Summary: human vastus lateralis pfi study



- Harmonization between labs is required (media, O₂ regime, pfi preparation ...). Controversial results have been found around the world.
- Human study (vastus lateralis) performed in Copenhagen between different labs (6 groups: Austria, Denmark, Germany, Spain, USA) to harmonize and/or find the best conditions for studies with muscle biopsies.
- Experimental design:
 - 3 biopsies ($N=3$, 1 biopsy/day) from the same volunteer.
 - Blinded study: 3 people in charge of the blinded study.
 - 4 media conditions (Buffer Z+/- Blebb; MiR05-Kit+/- Blebb) and two O₂ regimes: „normoxia“ (200-100 μ M) and hyperoxia (400-250 μ M)
 - $n=96$ pfi preparations (32/day). 2 people preparing pfi.
 - 4 people performing the experiments using High-Resolution Respirometry (SUIT protocol: SUIT-8 O₂ pfi D14) and data analysis.
- Preliminary results presented in Jurmala (MiP2018/MitoEAGLE Jurmala LV)

Human vastus lateralis pfi study. Next steps



- **Individual data analysis**
- **Define individual exclusion/analysis criteria (in parallel with the individual analysis)**
- **Retreat – analysis discussion. Stablish exclusion/analysis criteria (consensus)**
- **Re-analysis (final analysis) applying the commom criteria for exclusion/analysis**
- **Manuscript.**



WG2

MITO EAGLE data repository in muscle

Generating reference values on mitochondrial respiration in permeabilized fibers of mouse soleus muscle.



Summary of the discussion about ...

→ MitoEAGLE WG2 pilot study

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

→ Collection of data (Permeabilized fibers (pfi) - soleus)

Mouse model

- Mouse strain: C57BL6 J - Age: 14-20 weeks
- Gender: male (N=4) and female (N=4), total N=8
- **SUIT protocol: 1PM;2D;2c;3G;4S;5U;6Rot;7Ama**

→ Quick analysis of data

- Variability among groups (Flux)
- Same *FRC*

→ How to improve the protocol to reduce the variability?

- Additional labs
- Factors: *Ww*, mechanical permeabilisation (video, picture)
- Supply of chemicals to participating labs



Participants

Pilot study:

7 groups

Austria (Gnaiger's group)

France (Chabi's and Dubouchaud's groups)

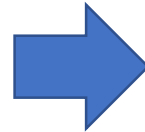
Germany (Klauss' and Roden's groups)

Spain (Garcia-Roves' group)

The Netherlands (Keijer's group)

and a STSM

(Carolina Doerrier)



Study phase 2:

8 groups

Canada (Lemieux's group)

Estonia (Puurand and Tepp's group)

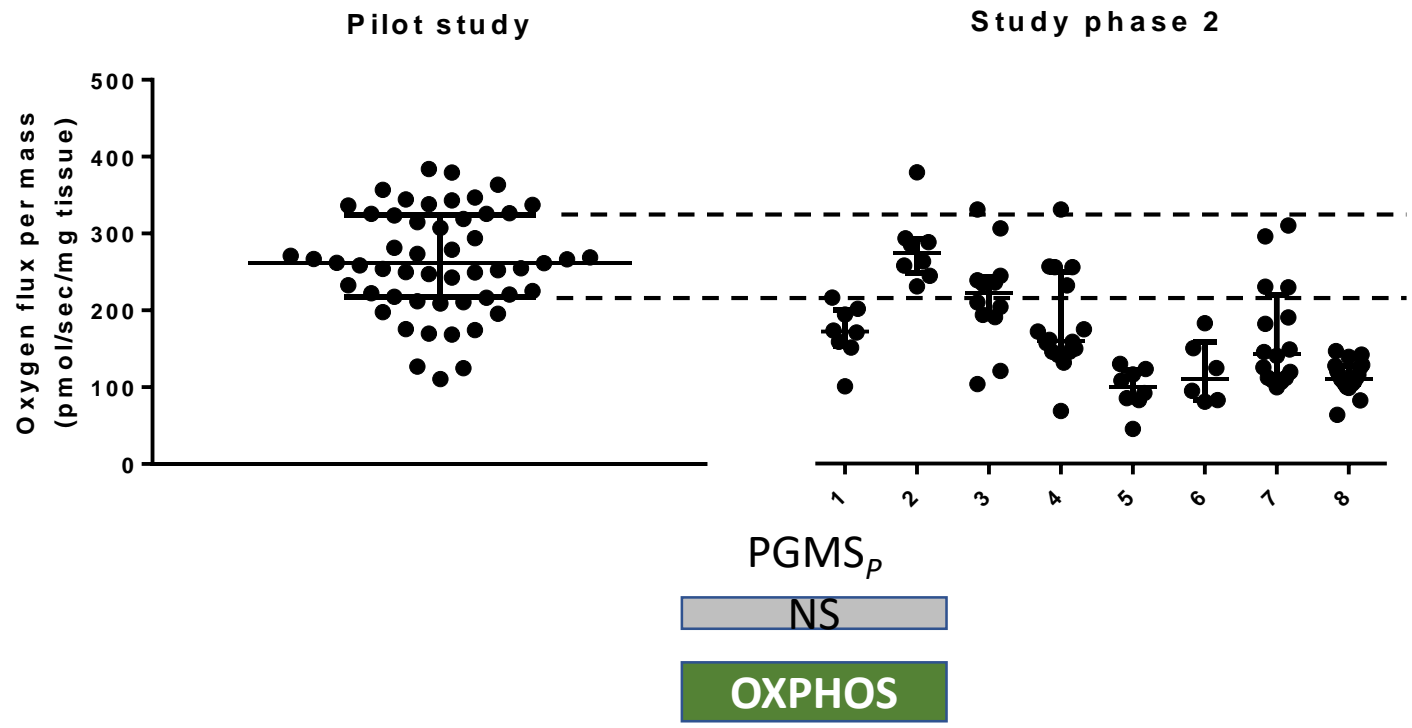
Italy (Calabria's group)

Spain (Acuña-Castroviejo's, Casado's and Villena's groups)

UK (Murray's group)

USA (Marcinek's group)

Preliminary results (without exclusion criteria)



Discussion

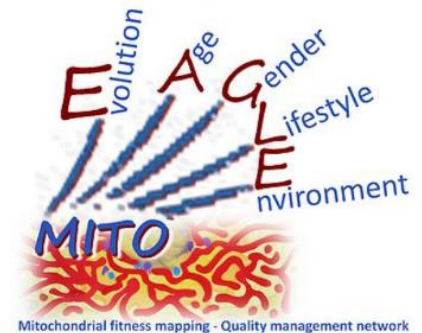
- Definition of exclusion criteria
- Intra-group variability
- Blotting of the skeletal muscle pfi specimen
- Weight scale resolution and quality of working station
- Platform (O2k and Hansatech, so far)
- Operator skills
 - Fiber preparation (pfi)
 - Experimental procedure and data analysis
- Data analysis – individual variability.
- Are we going to be able to discern among technical or personal skill performance and experimental variability?

- How to proceed?
 - Assignment of duties
 - Workshop

Metabolic signatures of human myotubes

Paper focusing on cellular fatty acid and glucose oxidation combining many cell studies with clinical data

- Age
 - Compare cells from very young to old subjects
- Gender
 - Compare cell data from male and female subjects from several studies
- Body weight
 - Cells from lean, overweight, obese, severely obese (SO) will be compared
- Exercise-status
 - Cells from athletic vs sedentary subjects (men only)
- Disease
 - Lean (normal) vs. obesity, IGT (prediabetes) and T2D
- *In vitro* vs. *in vivo*
 - Compare clinical data with metabolic cell data
 - Expression data may also be included (PCR, Western)
- Myotube interventions
 - Can be included ?



Metabolic signatures of human myotubes

- Example: combining cell data from sedentary (young and old) vs. athletes (retention of *in vivo* donor characteristics):
 - Cellular fatty acid and glucose oxidation correlated positively with VO_{2max} of the donors
 - Cellular fatty acid and glucose oxidation correlated negatively with the age of the donors
 - No correlations with body weight and BMI of the donors
- Donor group differences (lean vs. obese, young vs. old etc.) will also be studied
- Other Mitoeagle members should contribute with respirometry data (i.e. Seahorse data) from myotube studies?

WG2 Review: Myocardial mitochondrial dysfunction (altered function?) in obesity/diabetes

Country	Participant	Topic
Canada	Hélène Lemieux, Ted Han	Lifestyle intervention to target mitochondrial function
Estonia	Kersti Tepp, Marju Puurand, Tuuli Käämbre	Mitochondrial respiratory supercomplexes as regulators of mitochondrial performance
Latvia	Marina Makrecka-Kuka, Edgars Liepinsh	Metabolic flexibility in IR and diabetes Pharmacologic strategies to target mitochondrial dysfunction
Netherlands	Rob Wust; Paul de Goede	Metabolic flexibility in IR and diabetes; Regulation of mitochondrial function by circadian rhythm
Norway	Neoma T Boardman, Terje Larsen	Mitochondrial energetics in obesity/IR
Romania	Danina M. Muntean, Adrian Sturza	Role of MAO-related oxidative stress in diabetes
Turkey	Belma Turan, Erkan Tuncay, Yusuf Olgar	Metabolic flexibility in IR and diabetes Pharmacologic strategies to target mitochondrial dysfunction (MitoTEMPO)
UK	Andrew Murray, Katie O'Brien	Exercise training and cardiometabolic health

- Internal reviewers: Maria Luisa Genova (Italy); Maija Dambrova (Latvia); Carlos Palmeira (Portugal)
- Suggested journals: *Am J of Physiol (Endocrinology and Metabolism)*; *Physiology*; *Physiol Reviews*
- **Next Steps:**
 - Receiving prepared sections from contributors - till October 15th
 - Preparing first draft (Marina Makrecka-Kuka and Terje) - till October 30th
 - Feedback from contributors and internal reviewers
 - Finalizing manuscript for submission - provisional submission deadline January 2019
- ***Next plan for WG2 heart – review on methods to study cardiac mitochondrial function***