Isolation of PBMCs for High Resolution Respirometry and association to Basal Metabolic Rate

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11/16/2016

Enable Cluster “Healty food choices in all stages of life”

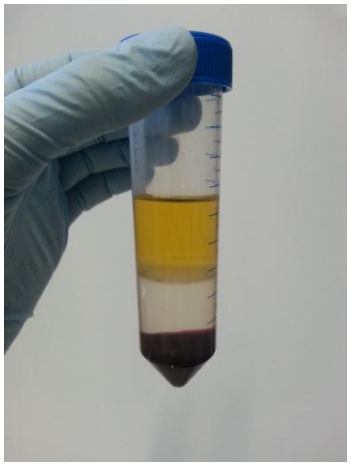
• Children	3 – 5 years	N = 100
• Adolescent	18 – 25 years	N = 100
• Adults	40 – 65 years	N = 200
• Seniors	75 years plus	N = 60

Blood samples for PBMC Isolation

Moreover:

- body composition and anthropometry
- Clinical analysis from blood samples
- Energy turnover
- Metabolomics
- Genetic and epigenetic
- Microbiota composition
- Nutritional habits
- Sensory systems

Isolation of PBMCs for High Resolution Respirometry



- Blood drawing: fasted between 9am and 10am

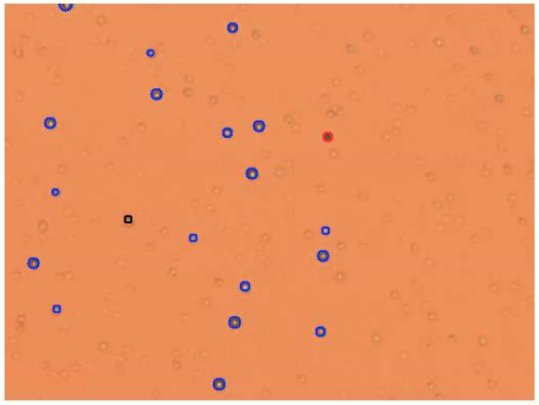
- 50 ml Falcon Tubes with 16 ml Ficoll
- 16 ml blood in PBS
- Diluted 1:1 (blood : PBS)
- Centrifugation: 400g, 25 min, accel 1, break 0

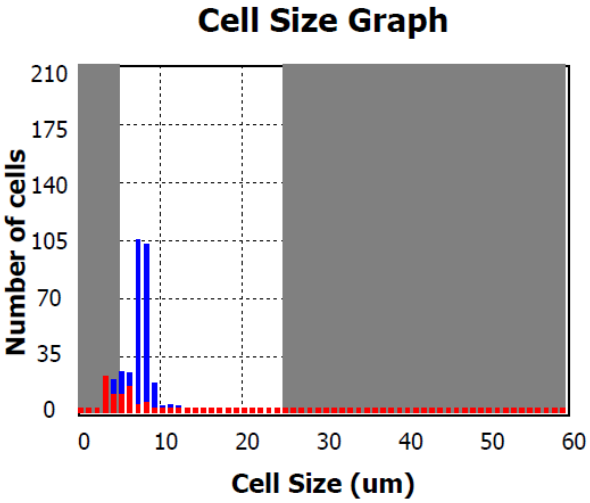
- Collection of PBMC layer by transfer pipettes
- Dilution with PBS
- Centrifugation 300g, 10 min, accel 5, break 9

- Discard supernatant, washing with 20 ml PBS
- Centrifugation 300g, 10 min, accel 5, break 9

Isolation procedure: 1 hour

Cell counting





Cell count

- Stained by Trypan blue
- Total, live, dead and viability of the cells
- Average viable cell size
- Average dead cell size

- No differentiation between PBMCs and Platelets

- **<u>Viability varies between 65 % - 95 %</u>**

Respiration Measurement

O2k chamber for HRR

- 6 Mill living cells/ chamber
- 100µl of the chamber is removed
- and 100µl of cell suspension is added to the chamber

Protocols – Permeabilized cells

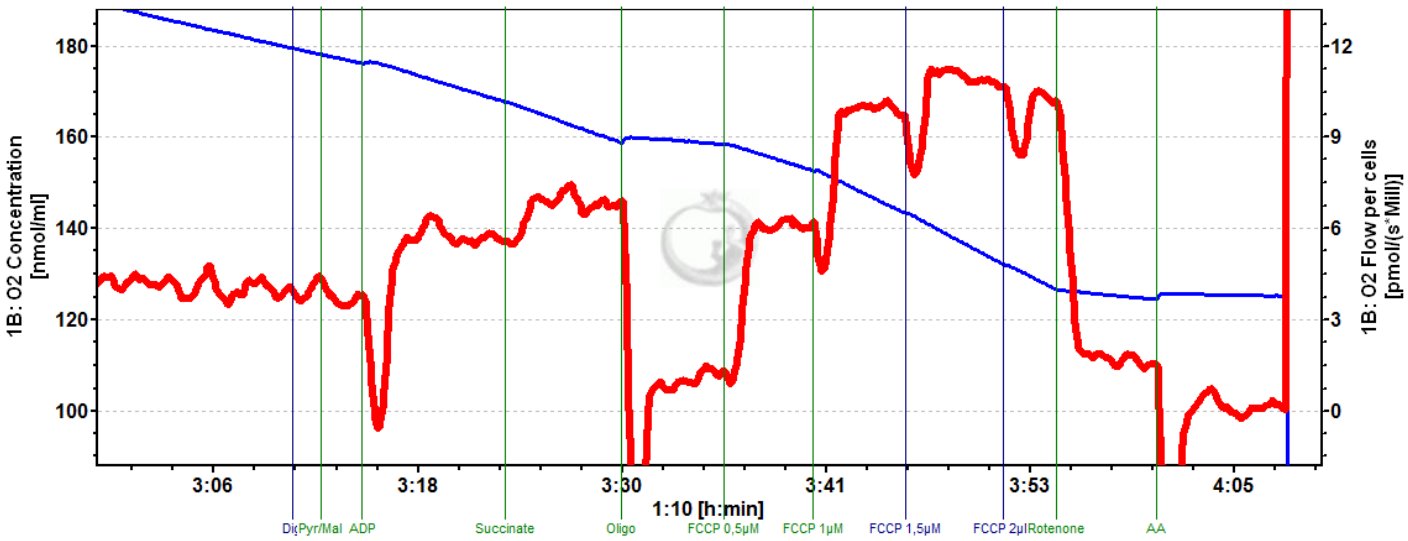
- Substrate Inhibitor Titration (SUIT) Protocol in Miro5
- Succinate and Rotenone in Miro5

Freezing subsample of suspension for later analysis

- PBMCs in FBS with DMSO for FACS Analysis
- PBMC pellet for enzyme activity and Western Blots
- Recovered cells of the respiration measurements for enzyme activity and Western Blots

Permeabilized PBMCs – SUIT Protocol

- Routine
- Digitonin
- Pyruvate & Malate
- ADP
- Succinate
- Oligomycin
- FCCP Titration
- Rotenone
- Antimycin A



Permeabilized PBMCs – Succinate and Rotenone

- Routine
- Digitonin
- Rotenone
- Succinate
- ADP
- Oligomycin
- FCCP Titration
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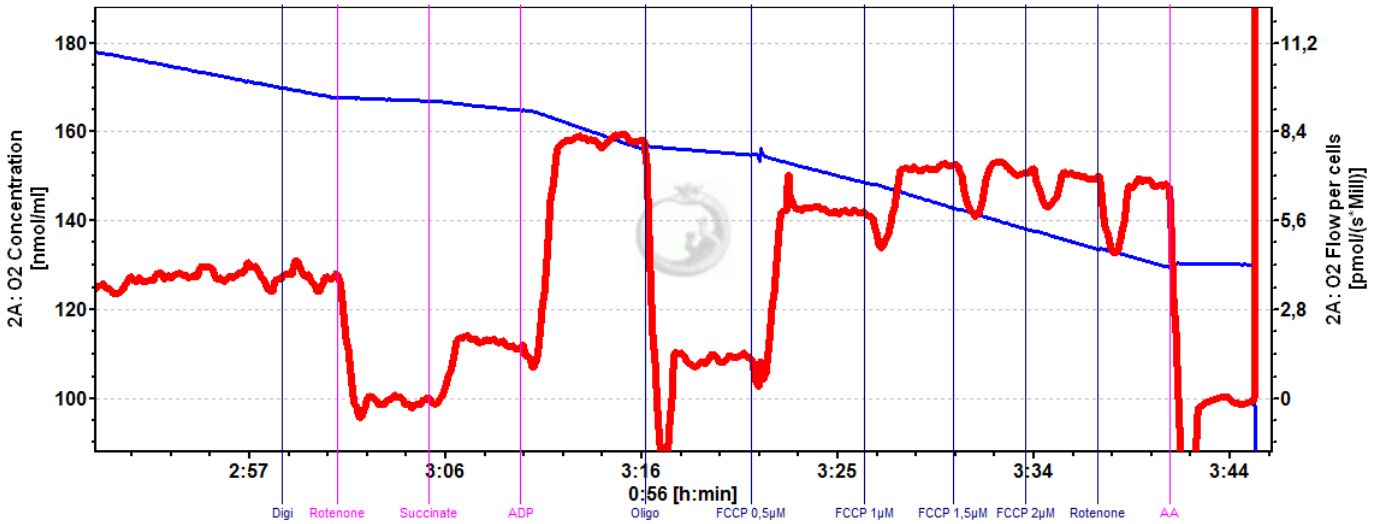
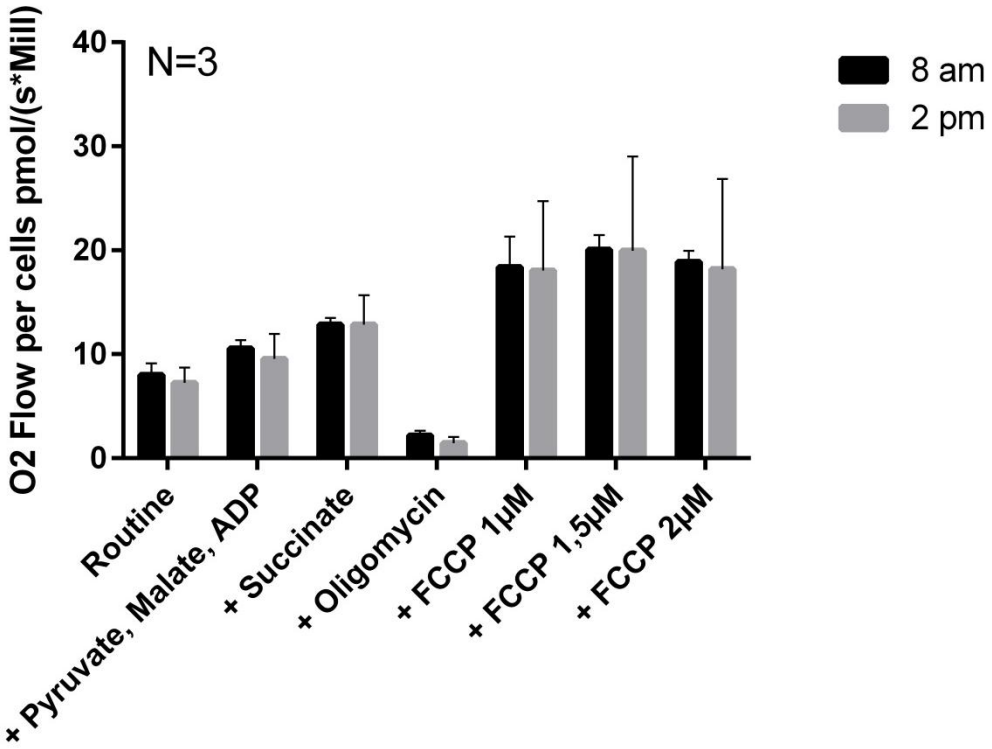


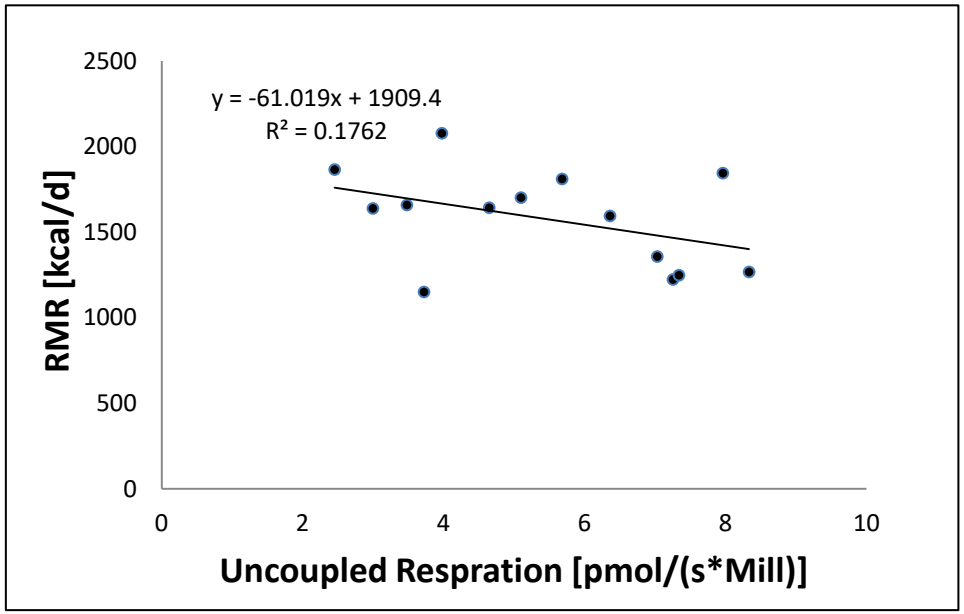
Time effect of PBMCs Isolation on the Respiratory Capacity



- Taking of blood sample at 8 am
- 1st isolation and respiration measurement at 8 am
- 2nd isolation and respiration measurement at 2 pm
- Blood sample stored in between on a walking frame at RT for 6 hours

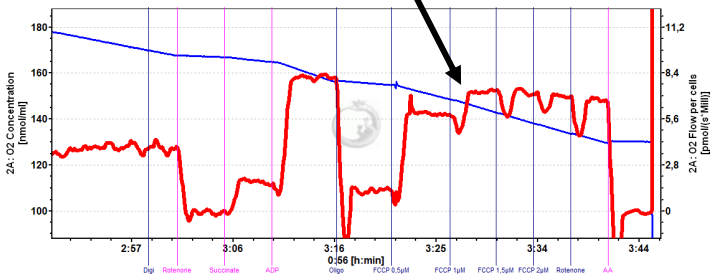
No decrease in the respiratory capacity of PBMCs after 6 hours!

Association of the respiratory capacity of PBMCs to the Resting Metabolic Rate



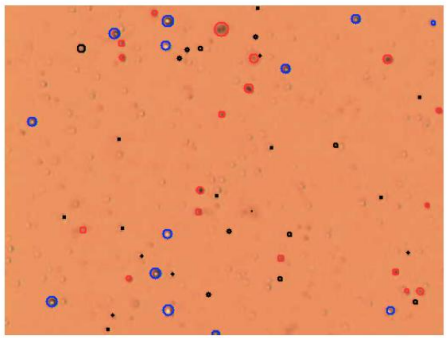
Uncoupled respiration by FCCP

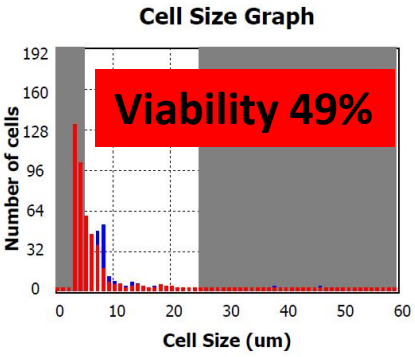
under the substrate of succinate

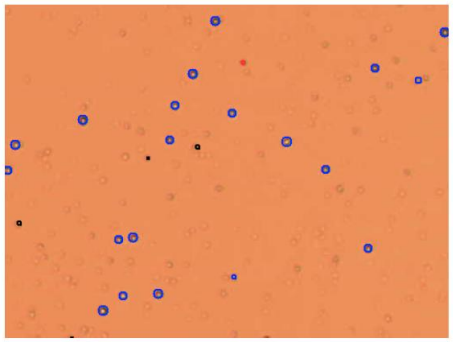


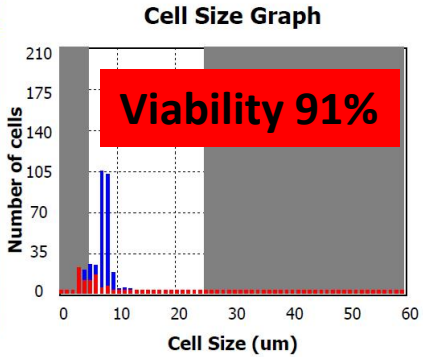
How to Deal with Dead Cells?

- Living cells are used for the respiration measurement
- At the end the results are related to million cells (living)
- But what if there are a lot of dead cells in a sample
  - Are they really dead?
  - Is the mitochondria still working and just the cell membrane disrupted?









Optimization Questions

- How to deal with dead cells
- Increasing the viability
- Difference in the cell number

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Dr. Yongguo Li,
Dr. Stefanie Maurer,
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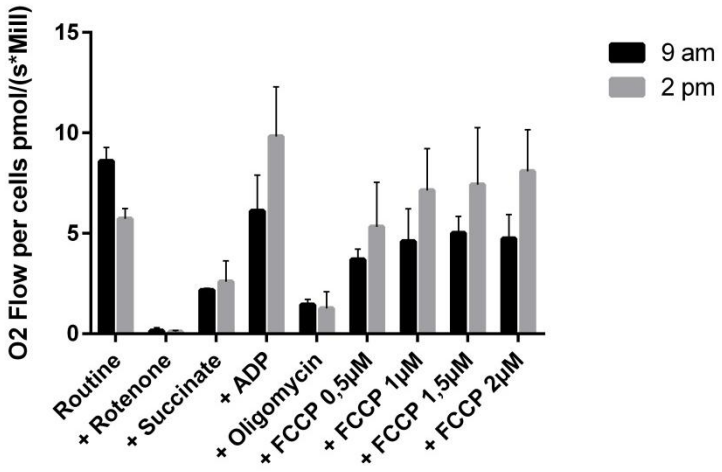
Gloria Keppner
Sabine Schweizer,
Hui Wang,
Sabine Mocek

enable
healthy food choices in all stages of life


O2 Flow per cells pmol/(s*Mill)
15
10
5
0
9 am
2 pm
Routine
+ Rotenone
+ Succinate
+ ADP
+ Oligomycin
+ FCCP 0,5µM
+ FCCP 1µM
+ FCCP 1,5µM
+ FCCP 2µM