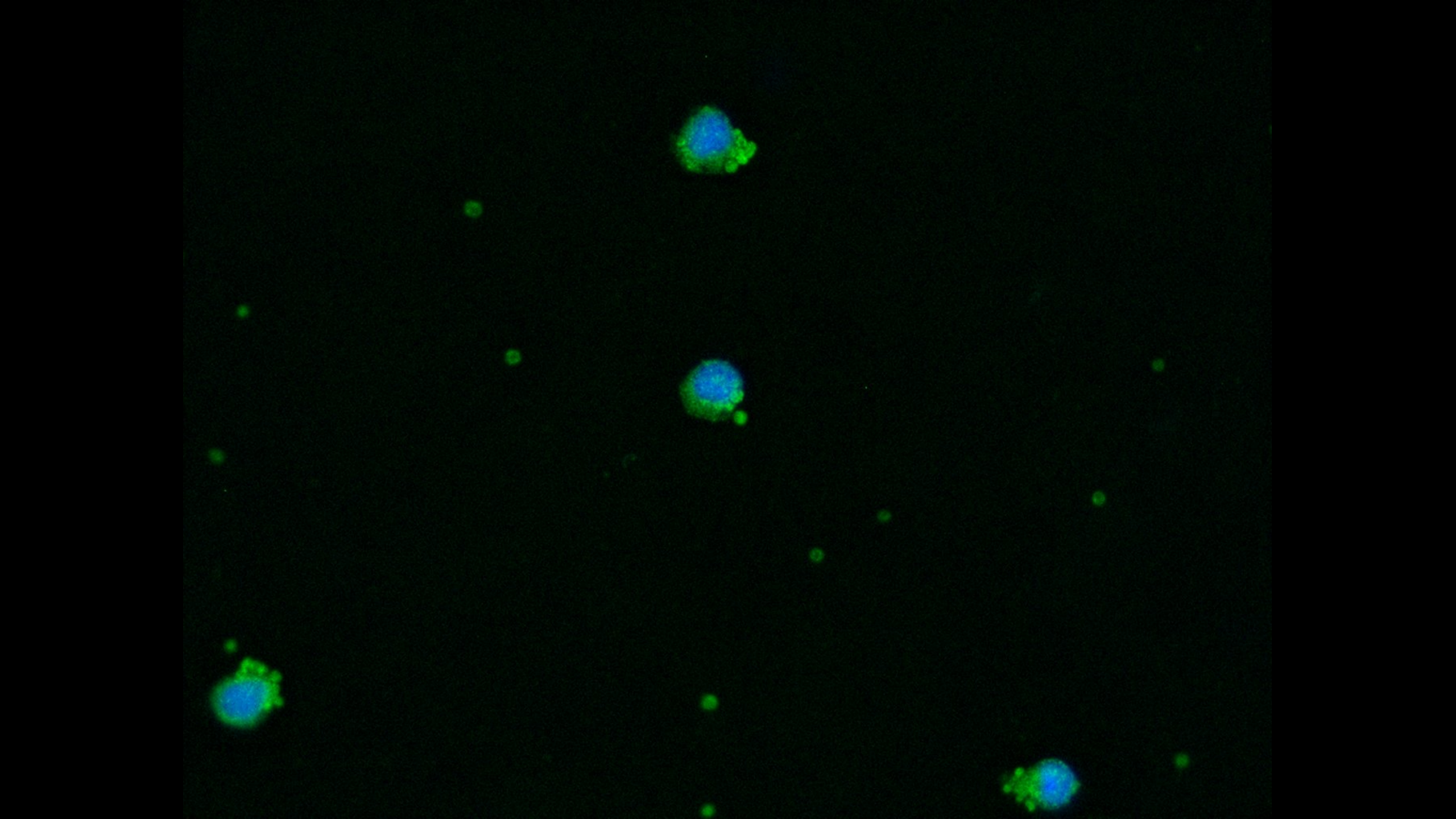

Human and rat PBMCs: what can we do better?

Verona, MitoEagle

November 15-17, 2016

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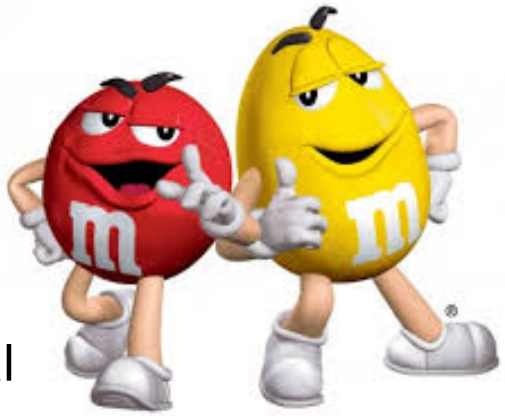
Nov 16, 2016

Sir. Material

Sir. Method

Sir. Material



Sir. Method

Sir. Doubt

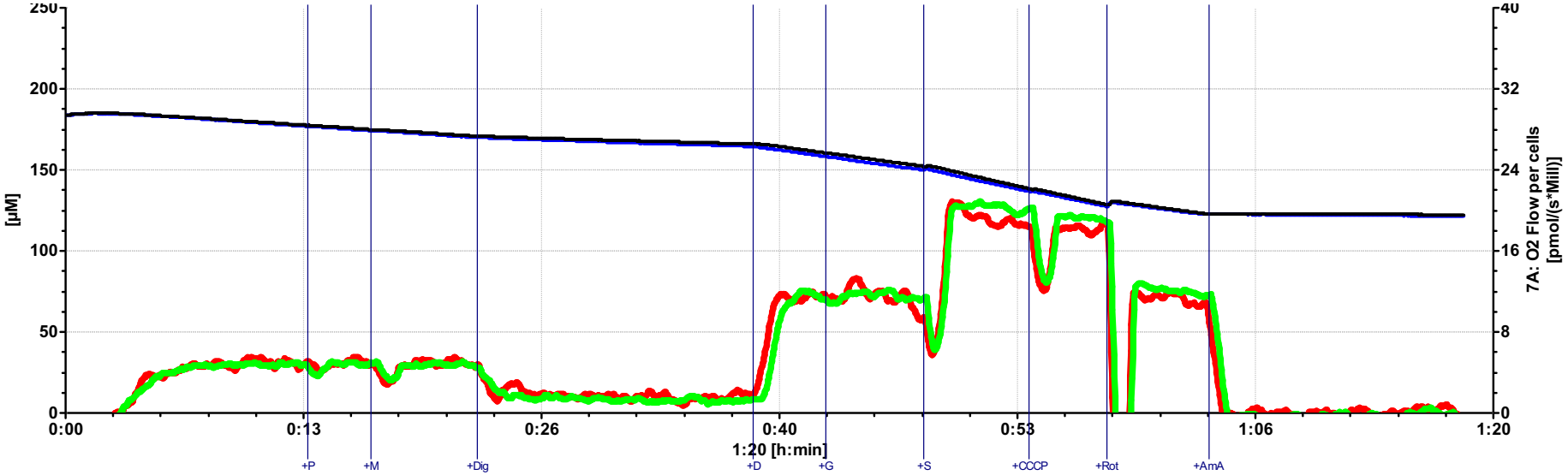
Material&Method
Isolation of PBMCs

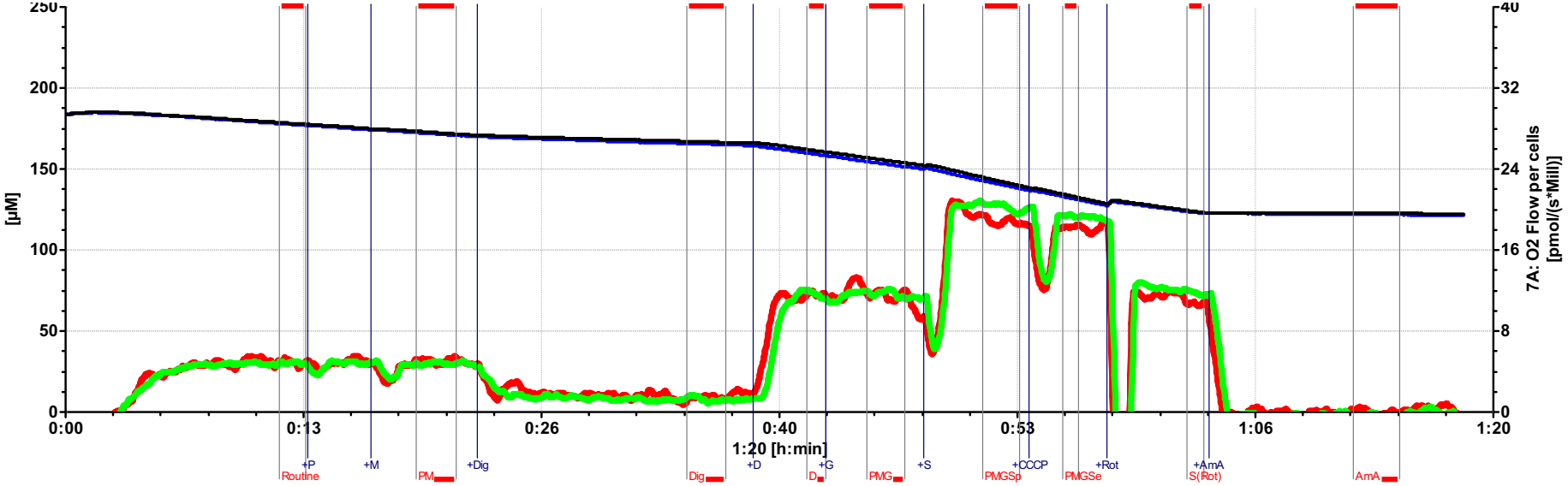
- Collection of **FRESH** human whole blood in EDTA-tubes
- Start of the isolation after 2hrs @RT
- Add 14.7 ml Ficoll into Leucoseptube (centrifuge 30 sec at 1000 *g*, RT). (Protect the tube with Ficoll to the light)
- Slowly pipette blood onto the Ficoll solution. Fill up to 50 ml with PBS
- Centrifuge for 30 min at 300 *g* RT without brakes (in the centrifuge: 9_|--- ---|_0)
- Collect PBMCs with a sterile Pasteur Pipette and transfer them in a new 50 ml Falcon tube. Fill up to 45 ml with PBS
- Centrifuge for 10 min at 300 *g* RT (in the centrifuge: 9_|--- ---|_9), RT. Discard the supernatant
- Wash with 20 ml PBS
- Count (2x10^6 cells/ml desired)
- Centrifuge for 10 min at 300 *g* RT (in the centrifuge: 9_|--- ---|_9), RT. Discard the supernatant
- Resuspend the pellet (2x10^6 cells/ml) in MiR05
- Assessment of mitochondrial activity by high resolution respirometry

Substrates/ Inhibitors	Injected volume µl	Concentration	Final Concentration
	Wait for stable plateau		
Pyruvate	5	2 M	5 mM
Malate	10	0.4 M	2 mM
Digitonin	4	10 mg/ml	0.02 mg/ml
	Wait for stable plateau (at least 10 min)		
ADP	4	0.5 M	1 mM
Glutamate Wait a bit	5	2 M	5 mM
Succinate	20	1 M	10 mM
CCCP	1	1 mM	0.5 mM
Rotenone	4	1 mM	2 µM
	Wait for stable plateau		
Antimycin A	0.5	5 mM	1.25 µM

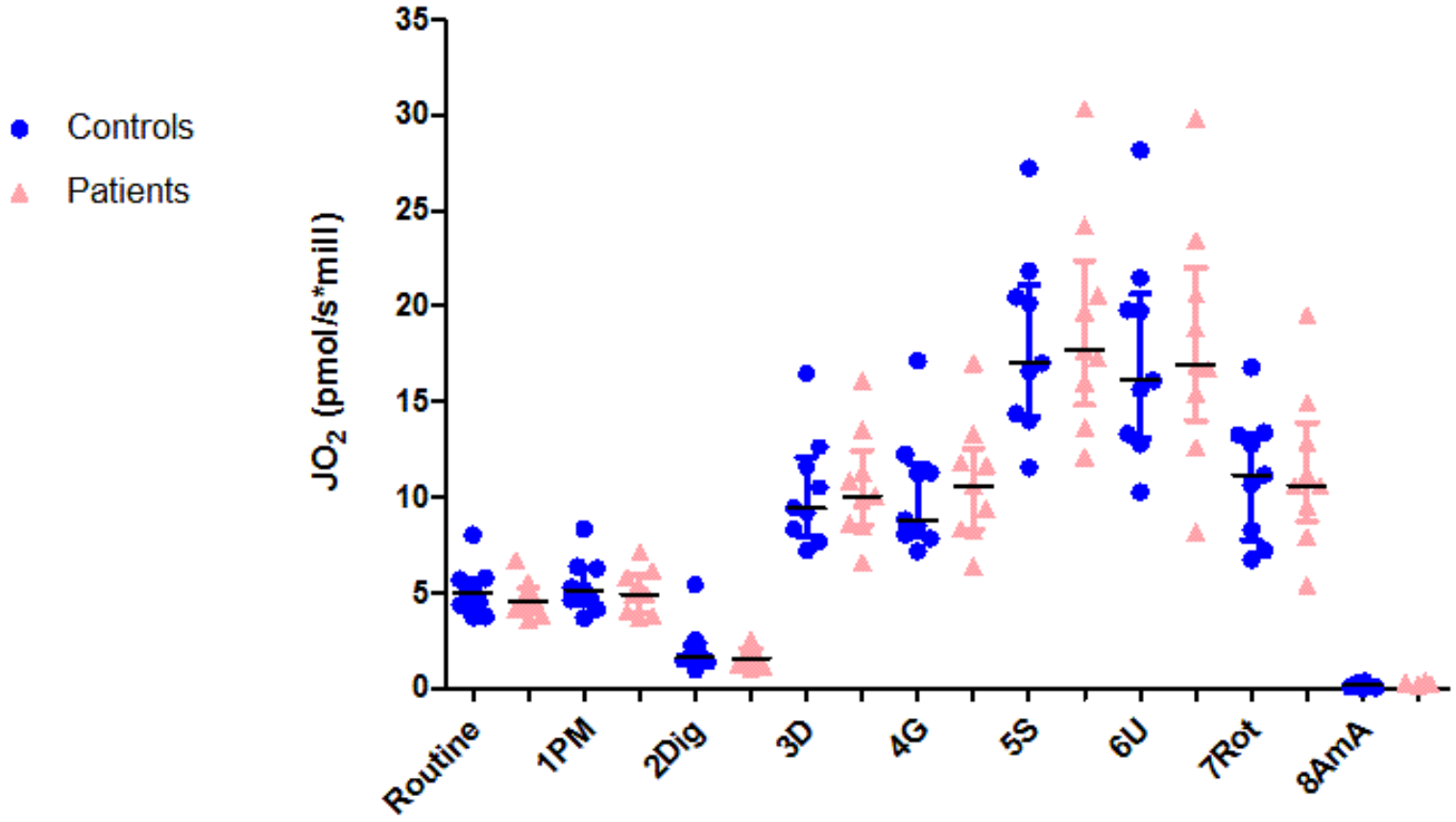
Material&Method

HRR traces

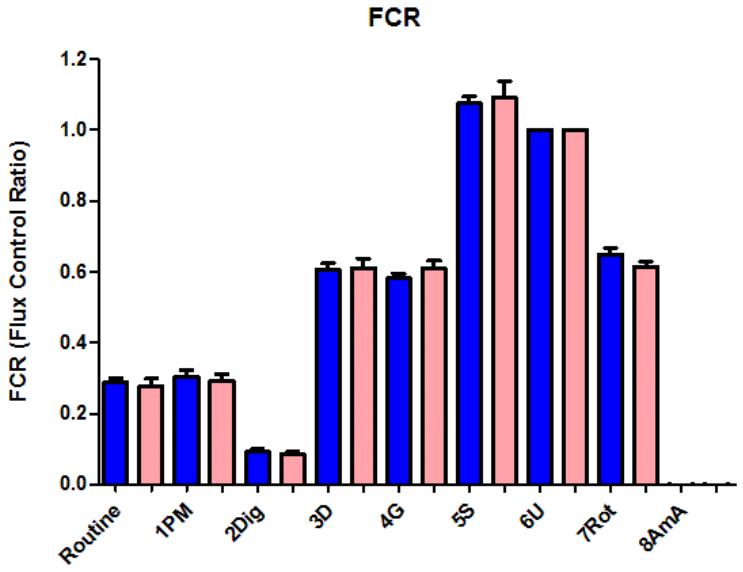




Isolated PBMCs mitochondrial respiration between Control (*blue*, *n=9*) and Patient (*pink*, *n=9*) subjects



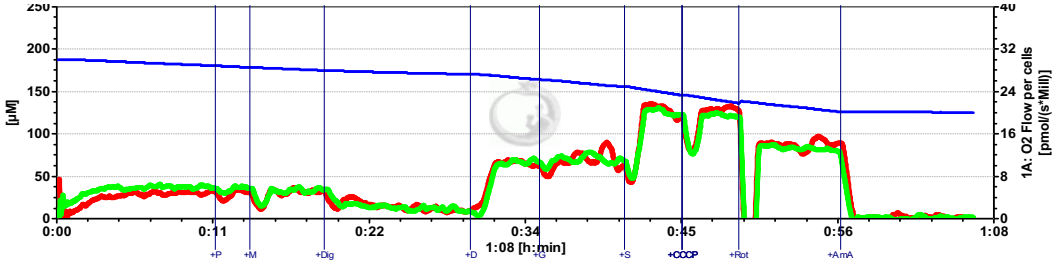
Statistics: Data plotted as median with interquartile range



Statistics: Data plotted as mean with SEM

Method&Doubts

110815-01, C



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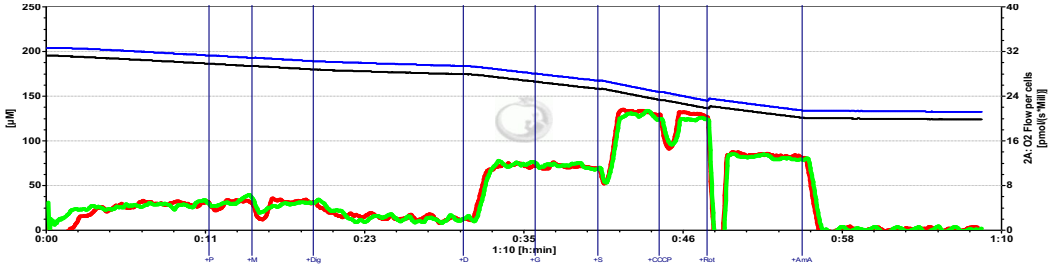
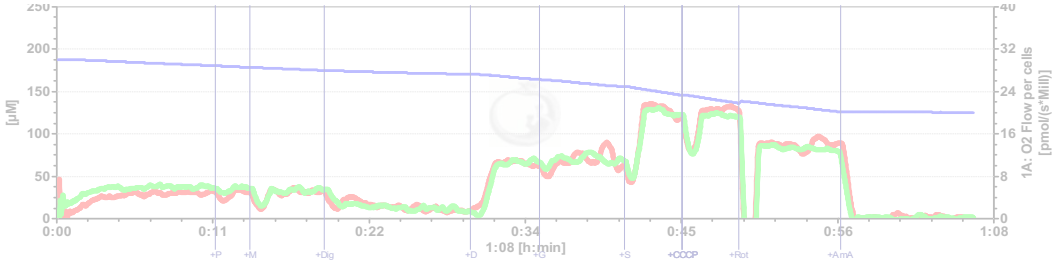


But …

Method&Doubts

110815-01, C



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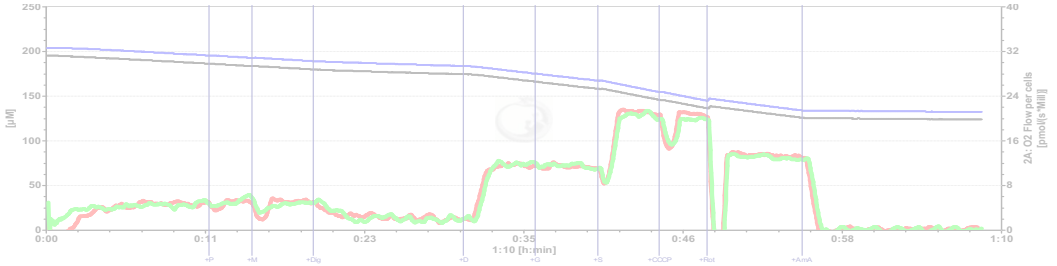

But ...

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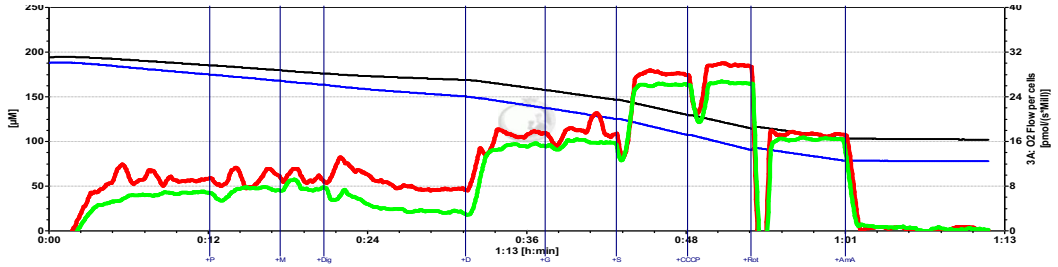

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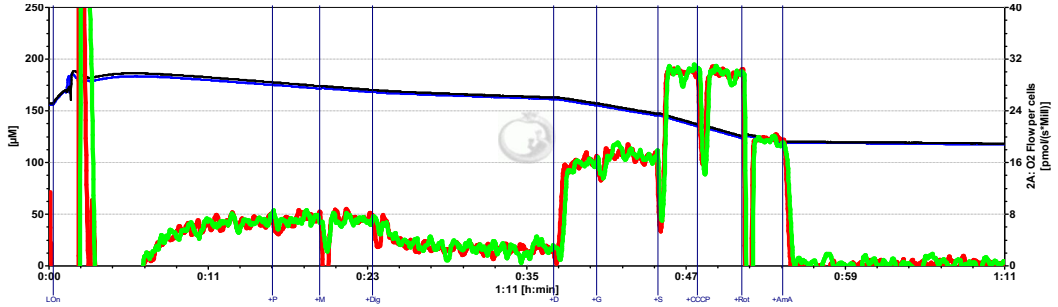
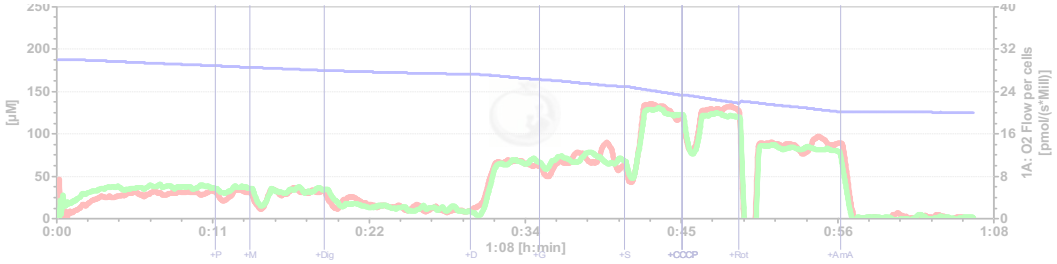


Method&Doubts

110815-01, C



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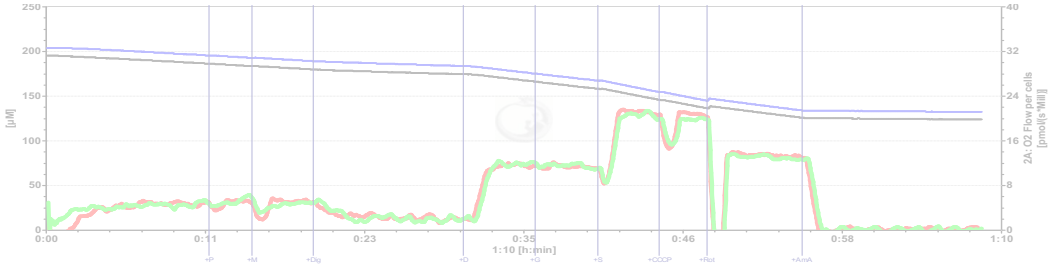

But …

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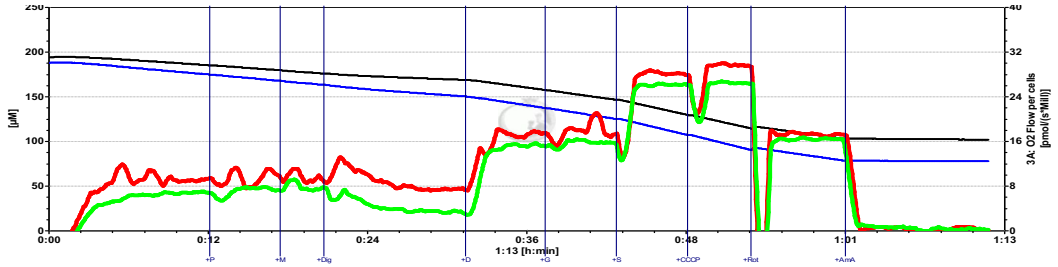

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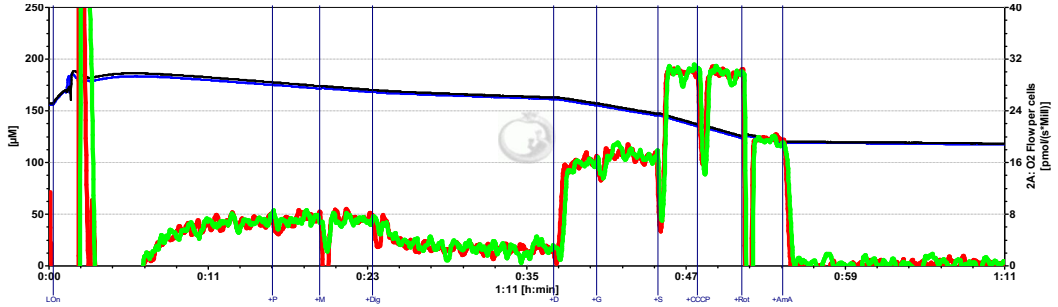

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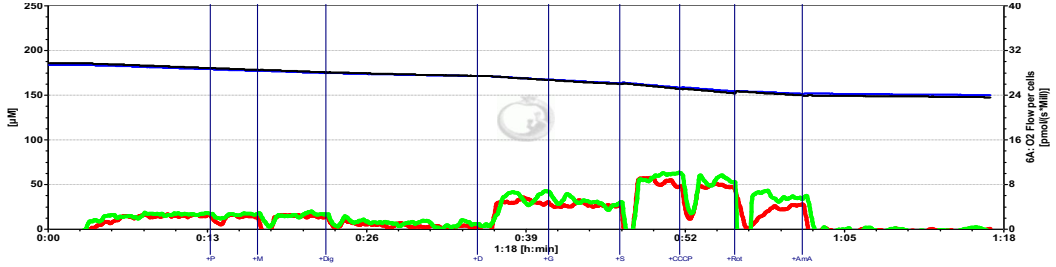
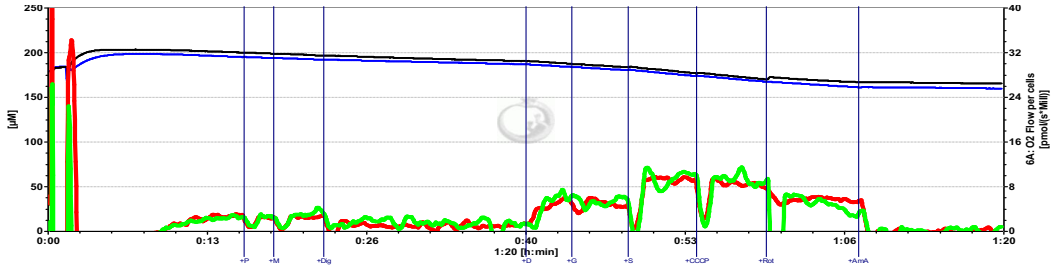
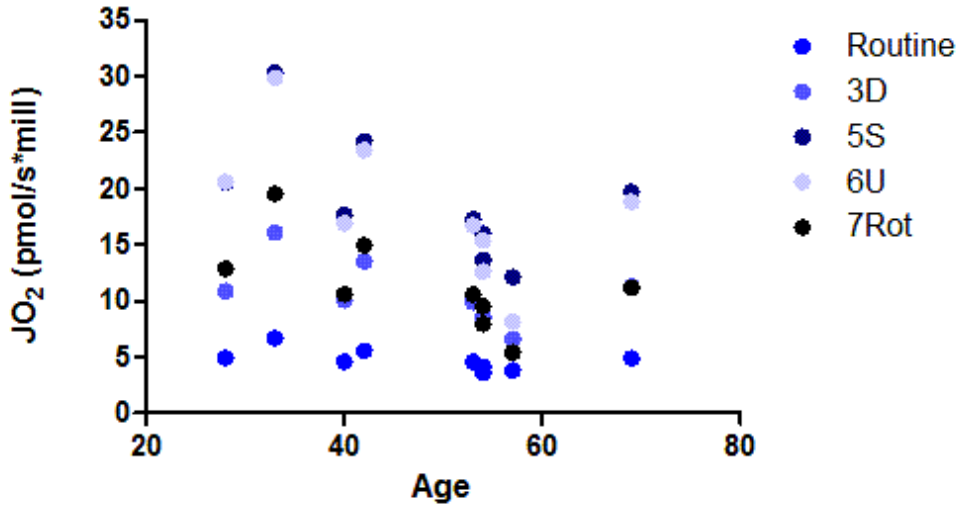
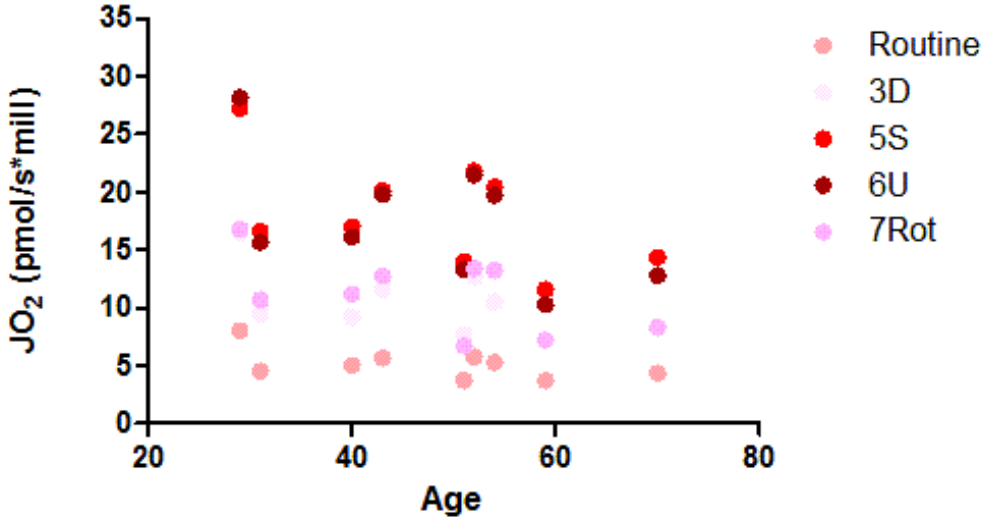


110915-18, P

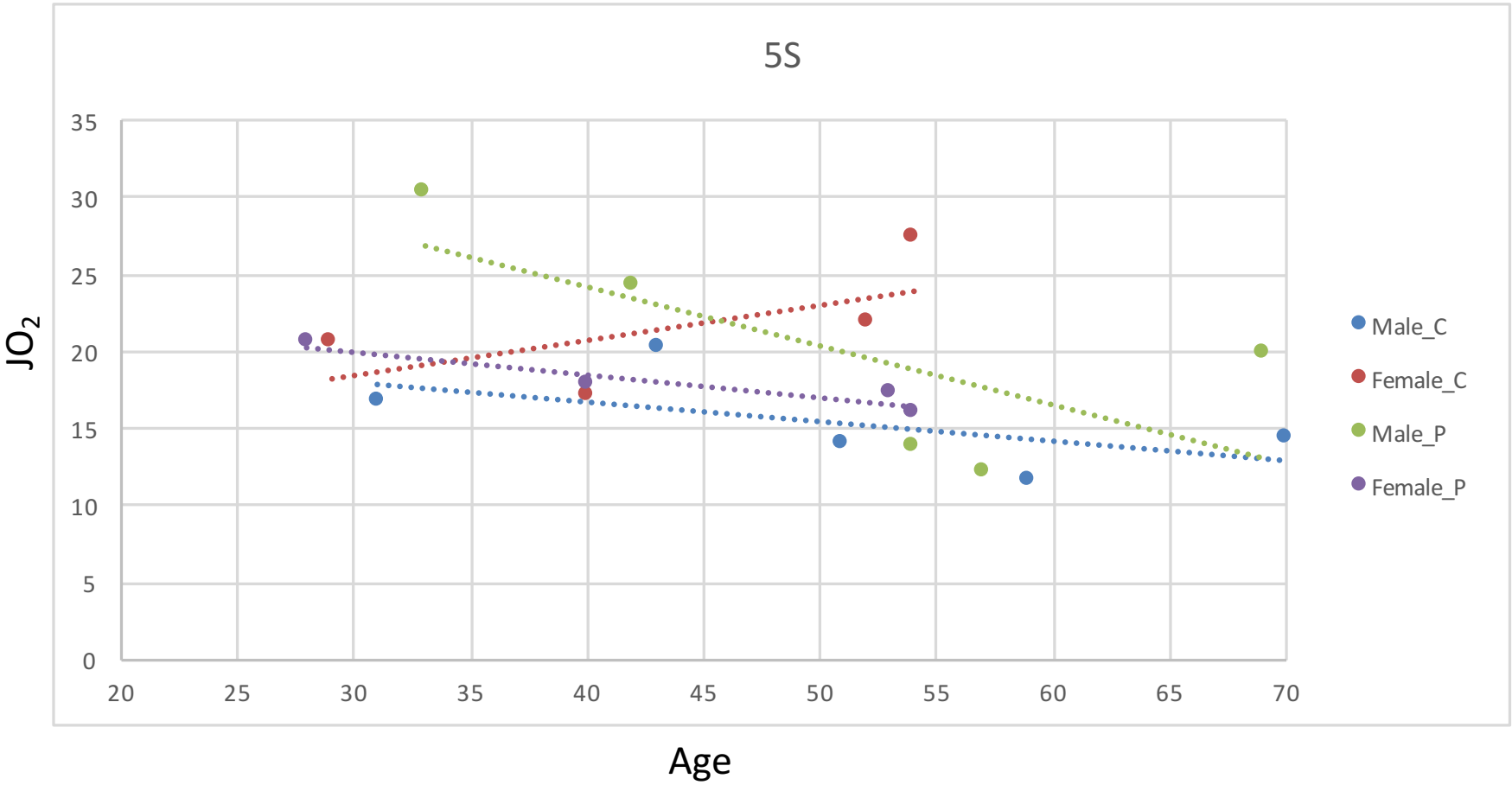


PBMCs Mitochondrial Respiration declines with age





PBMCs Mitochondrial Respiration and the Gender Effect



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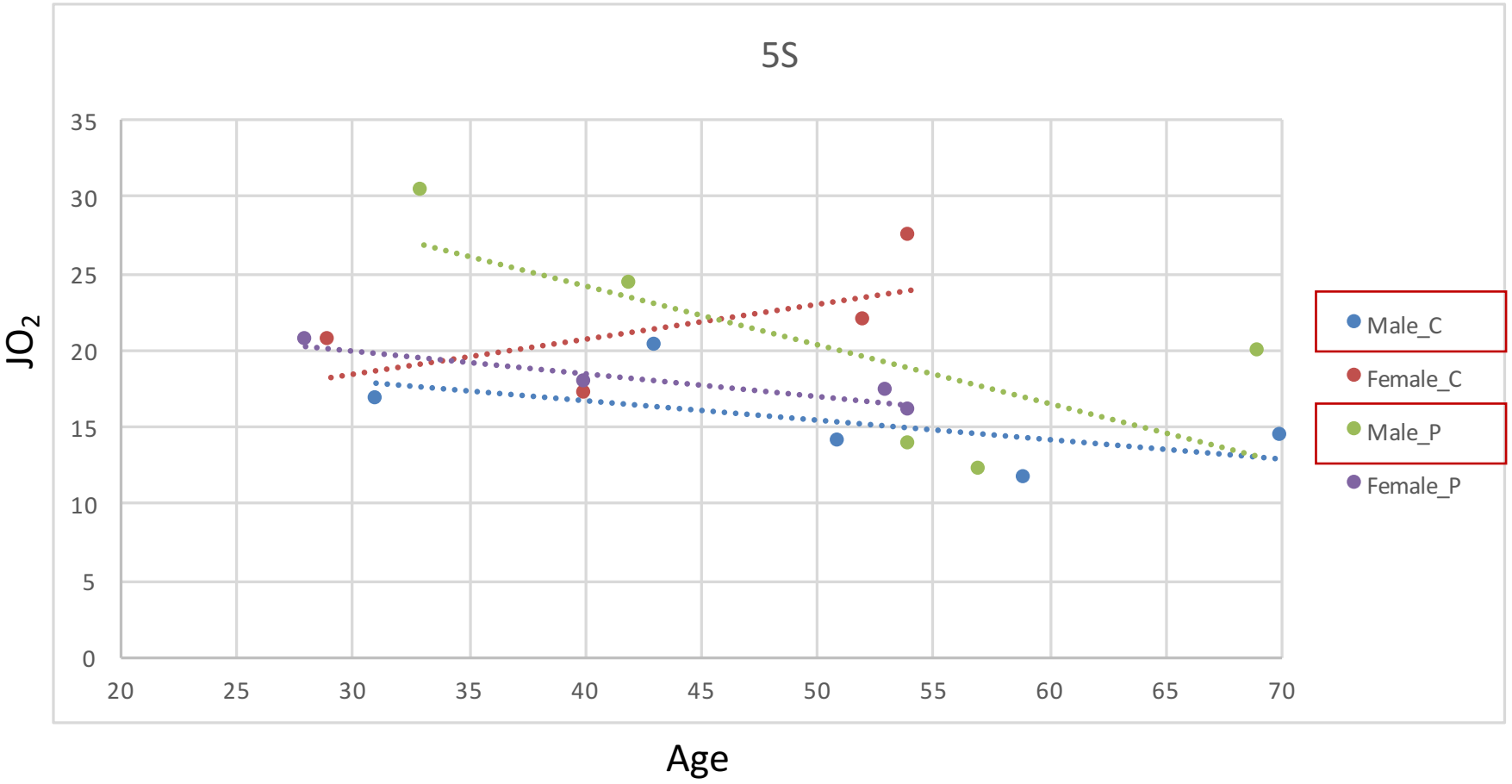

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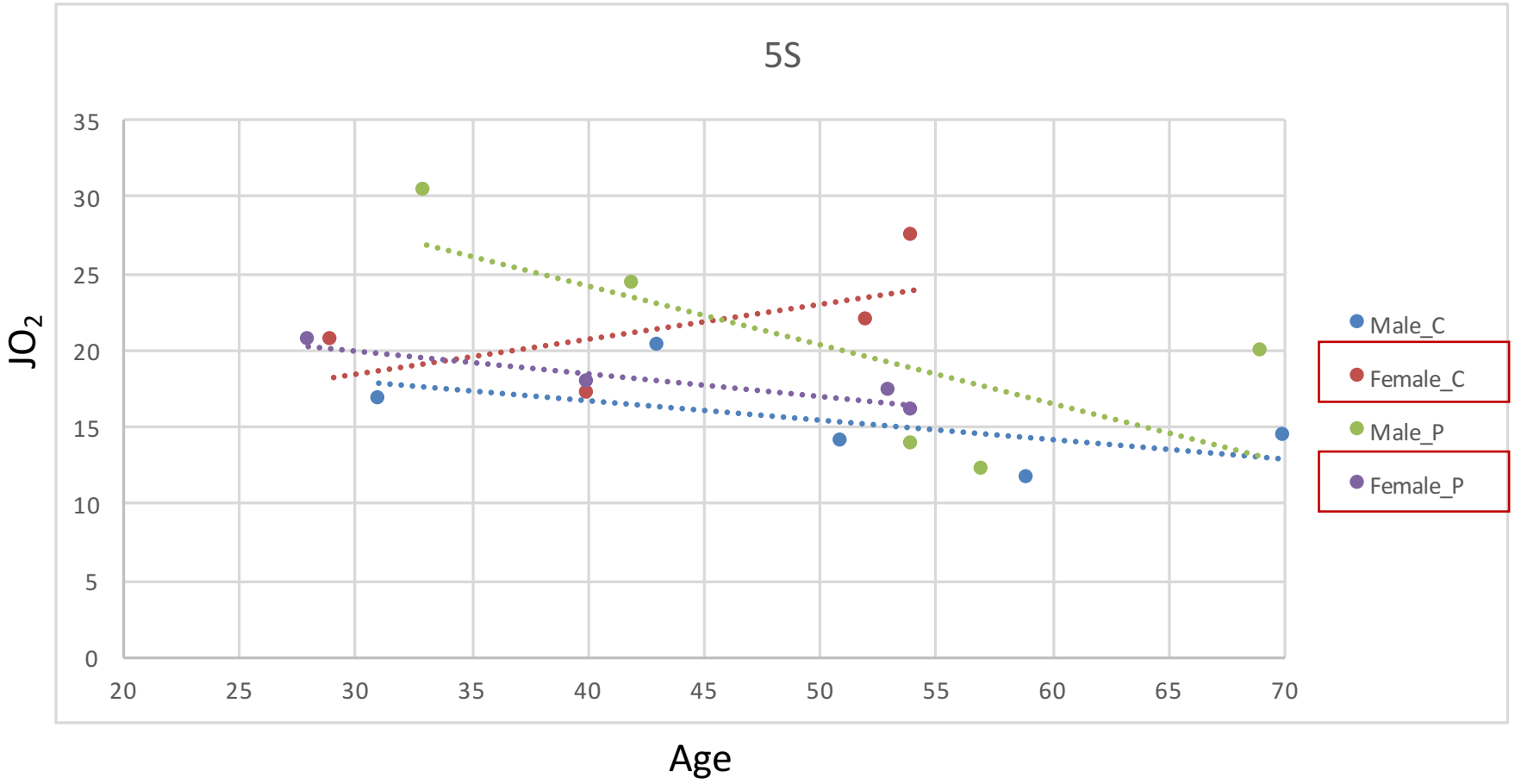


Material&Method
Isolation of rat PBMCs

5 ml Ficoll-Paque PREMIUM 1.084 into Falcon tube for 9ml whole blood

Slowly pipette blood onto the Ficoll solution

Centrifuge for 30 min at 300xg without brakes

Collect PBMCs with a sterile Pasteur Pipette or a 10ml Pipette and transfer them in a new 50 ml Falcon tube,

Wash with RPMI-1640

Centrifuge for 10 min at 300 xg

Discard the supernatant

Wash with RPMI-1640

Centrifuge for 10 min at 300 xg

Resuspend in desidered amunt of RPMI-1640

Count the cells

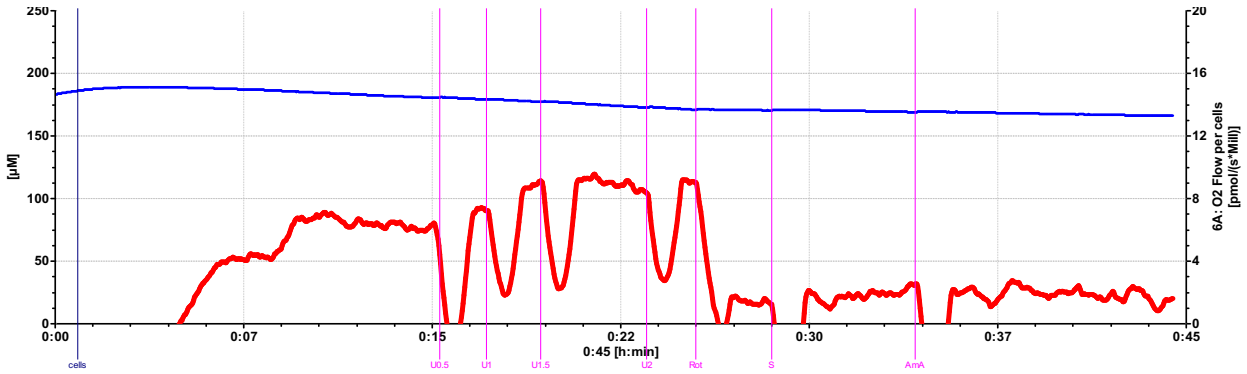
Desidered amount of cells: 2*10^6 cells/ml

(no less than 1*10^6 cells/ml)

Rat PBMCs isolation and HRR traces

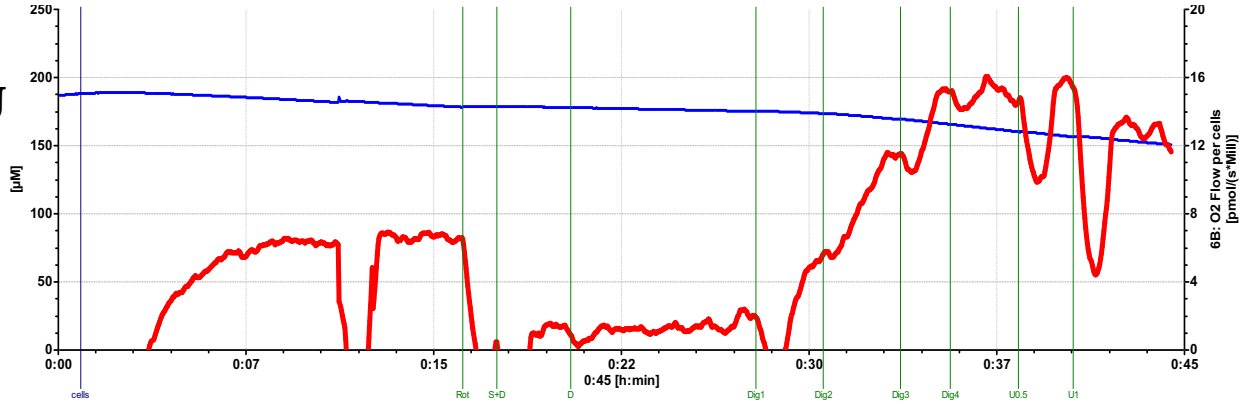
R 1U 2Rot 3S 4Ama

2*10^6 cells/ml



R 1Rot 2S 3D 4Dig-titr 5U

2*10^6 cells/ml



Thank you for the attention

KEEP
CALM
AND
EAT
M&M`S