MitoEAGLE data repository: adipose, liver, neuronal

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Katie Brooks

Deliverables

- Guidelines for future research and recommendations for the evaluation of respiratory characteristics in fat tissue.
- A database on mt fitness evaluated with fat tissue from humans and model organisms and post-study reports
- Draft of review manuscript.

Fat tissue analysis from human in comparison with mouse

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Departure: Wednesday, February 21st

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Ongoing project 2017-2018

COST support ?

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COST support ?

Flow Cytometry of Mouse and Human Adipocytes for the Analysis of Browning and Cellular Heterogeneity

Authors Carolina E Hagberg, Qian Li, Maria Kutschke, Debajit Bhowmick, Endre Kiss, Irina G Shabalina, Matthew J Harms, Olga Shilkova, Viviana Kozina, Jan Nedergaard, Jeremie Boucher, Anders Thorell, Kirsty L Spalding

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Description Adipocytes, once considered simple lipid-storing cells, are rapidly emerging as complex cells with many biologically diverse functions. A powerful high-throughput method for analyzing single cells is flow cytometry. Several groups have attempted to analyze and sort freshly isolated adipocytes; however, using an adipocyte-specific reporter mouse, we demonstrate that these studies fail to detect the majority of white adipocytes. We define critical settings required for adipocyte flow cytometry and provide a rigid strategy for analyzing and sorting white and brown adipocyte populations. The applicability of our protocol is shown by sorting mouse adipocytes based on size or UCP1 expression and demonstrating that a subset of human adipocytes lacks the β_2-adrenergic receptor, particularly in the insulin-resistant state. In conclusion, the present study confers key technological insights for analyzing and sorting mature …

- **Guidelines for future research and recommendations for the evaluation of respiratory characteristics in fat tissue.**

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1) High resolution respirometry Oroboros (diluted mitochondria in suspension)

Vs

Seahorse
(compacted mitochondria in layer)

Any comments? Send to me!

2) Thermogenesis as oxygen consumption

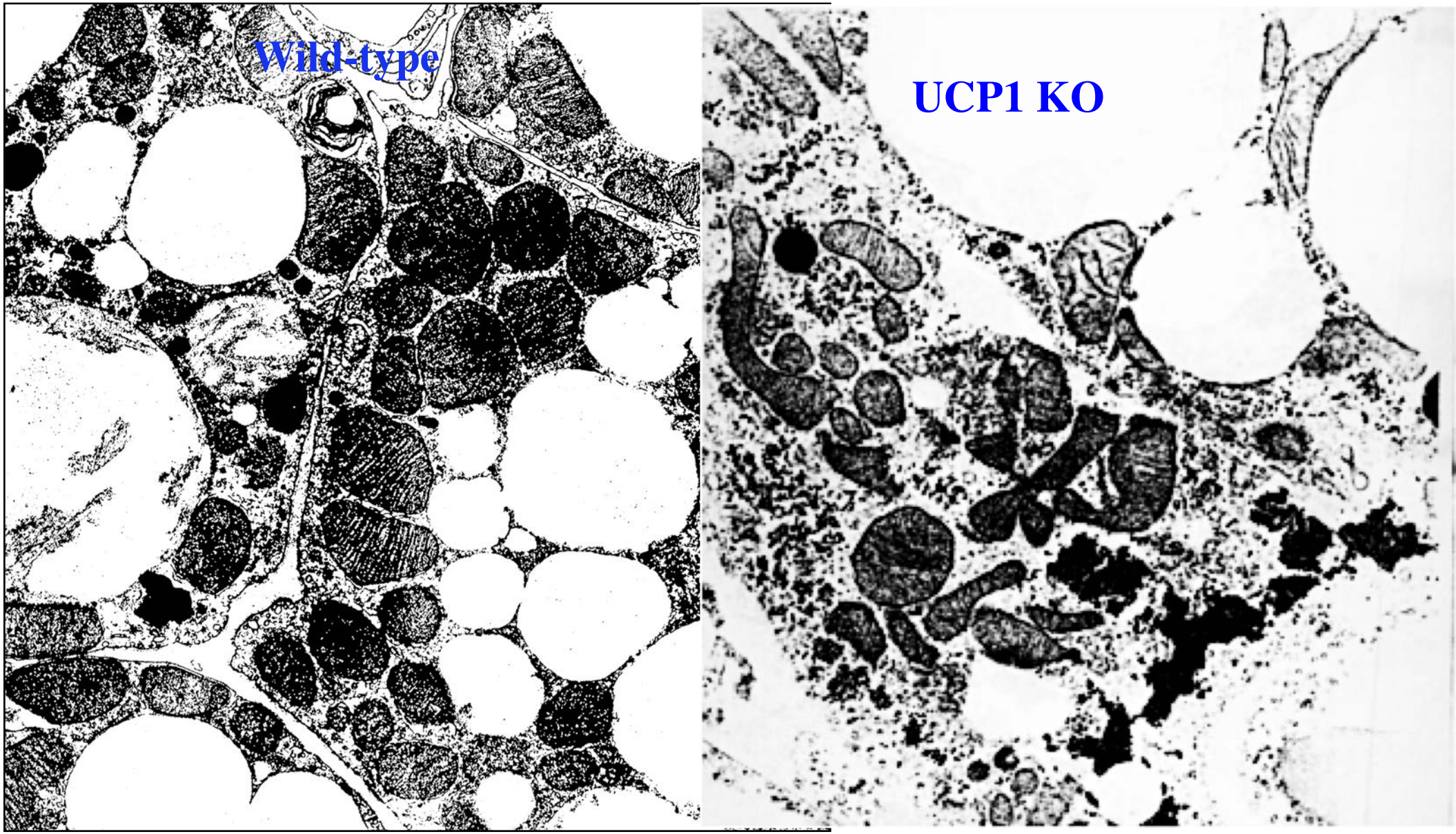
vs

Thermogenesis as heat production

SymCel the first isothermal microcalorimetry instrument

- **Guidelines for future research and recommendations for the evaluation of respiratory characteristics in fat tissue.**

C57Bl/6 mice
Room temperature



A. Giordano, V. Golozubova, S. Cinti - unpublished

Optimal medium for respiration of brown-fat mitochondria

Sucrose medium

Normal tonicity

250 mM sucrose
4 mM KPi
20 mM K^+TES
1 mM EDTA
2 mM $MgCl_2$
0.1-0.3% BSA

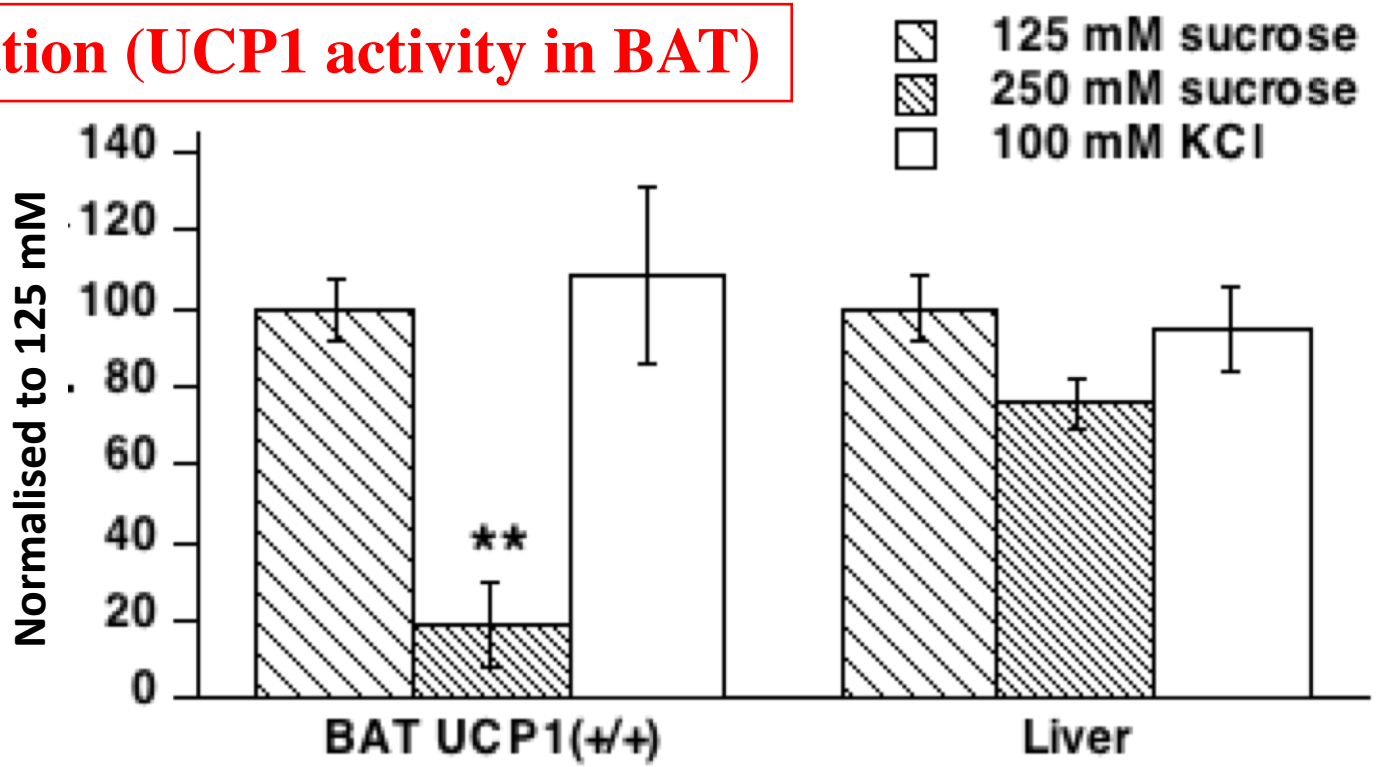
Low tonicity

125 mM sucrose
4 mM KPi
20 mM K^+TES
1 mM EDTA
2 mM $MgCl_2$
0.1-0.3% BSA

KCl medium

100 mM KCl
4 mM KPi
20 mM K^+TES
1 mM EDTA
2 mM $MgCl_2$
0.1-0.3% BSA

Substrate oxidation (UCP1 activity in BAT)



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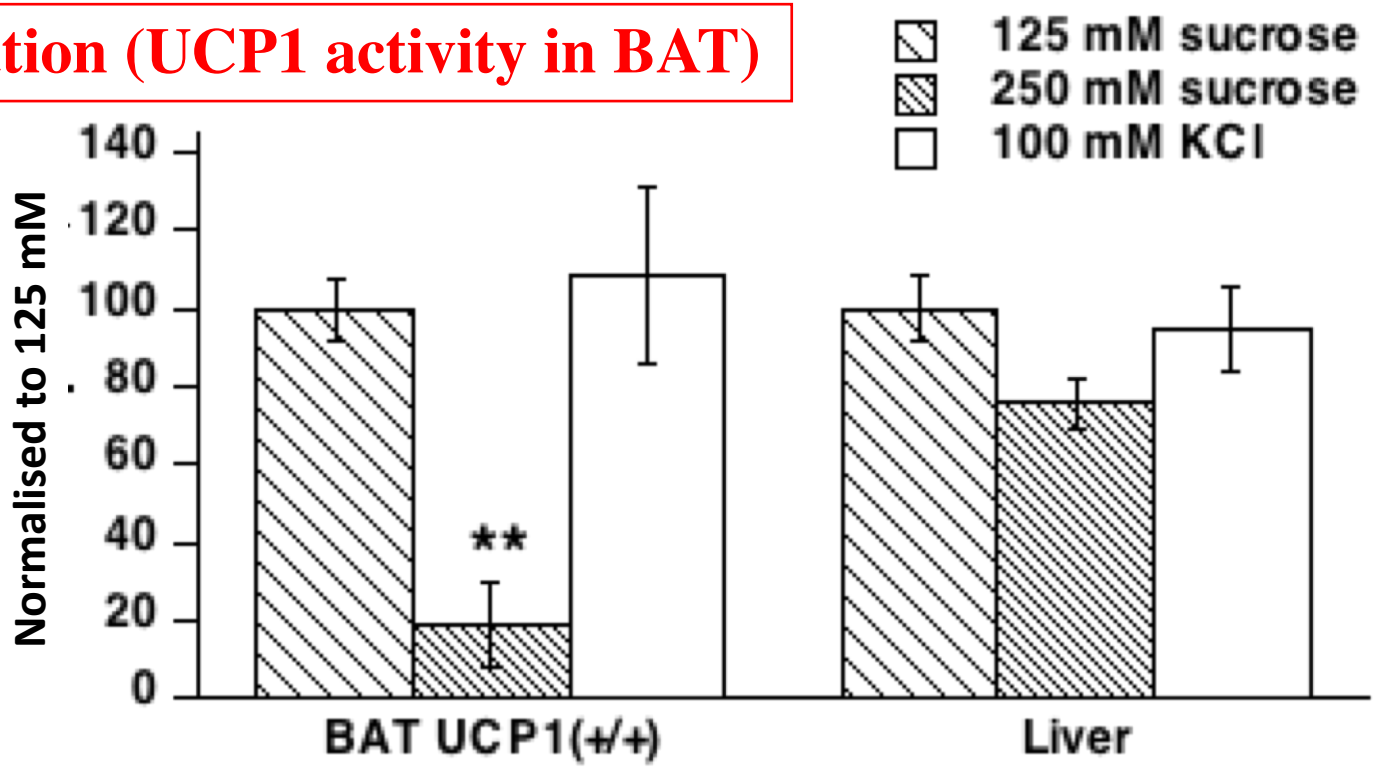
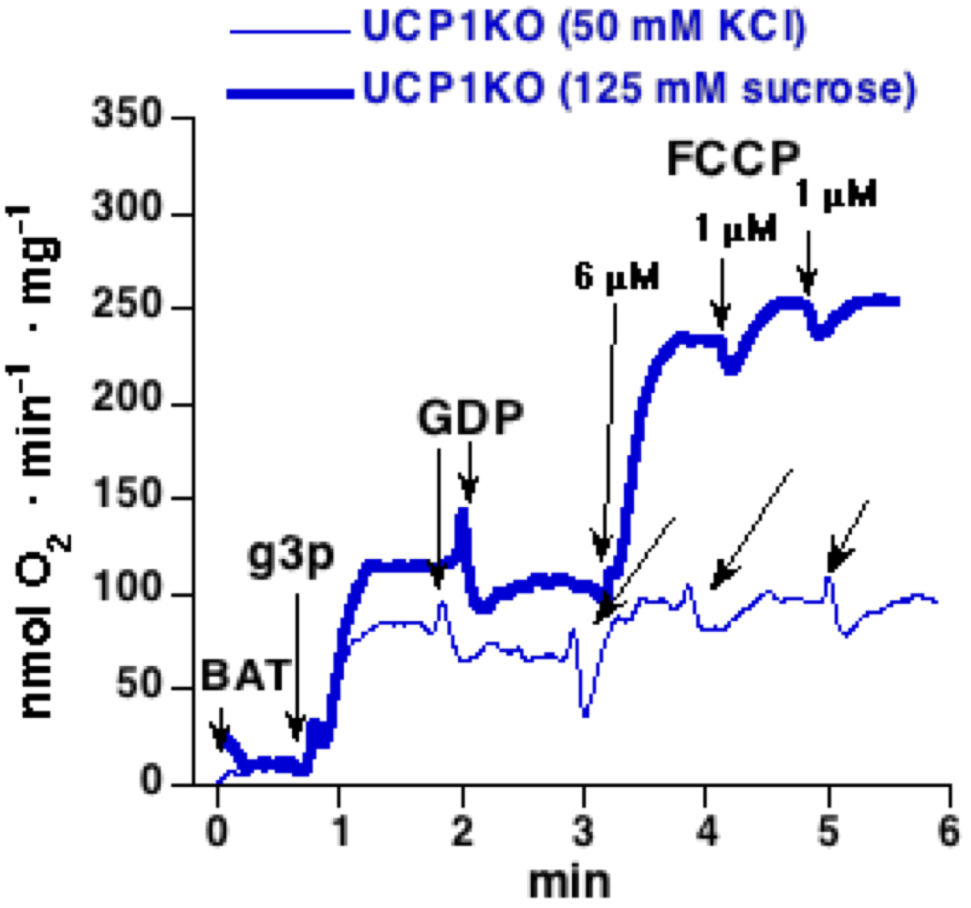
20 mM K^+TES
1 mM EDTA
2 mM $MgCl_2$
0.1-0.3% BSA

Low KCl medium

50 mM KCl
4 mM KPi
5 mM Hepes
1 mM EGTA
2% BSA

Substrate oxidation (UCP1 activity in BAT)





Low KCl medium

50 mM KCl
4 mM KPi
5 mM Hepes
1 mM EGTA
0.5% BSA

Low tonicity sucrose medium

125 mM sucrose
4 mM KPi
20 mM K+TES
1 mM EDTA
2 mM MgCl2
0.5% BSA

Shabalina IG, …..... Cannon B. Biochim Biophys Acta. 2010 1797(6-7):773-84.

UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic
IG Shabalina, N Petrovic, JMA de Jong, …J Nedergaard - Cell reports, 2013

BEIGE/BRITE MITOCHONDRIA

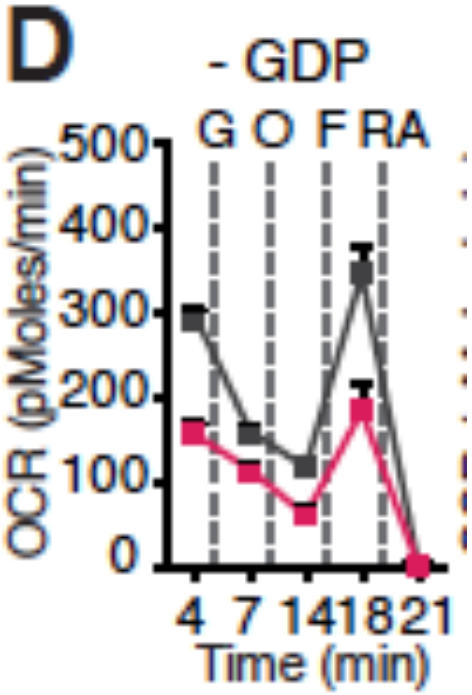
Low KCl medium

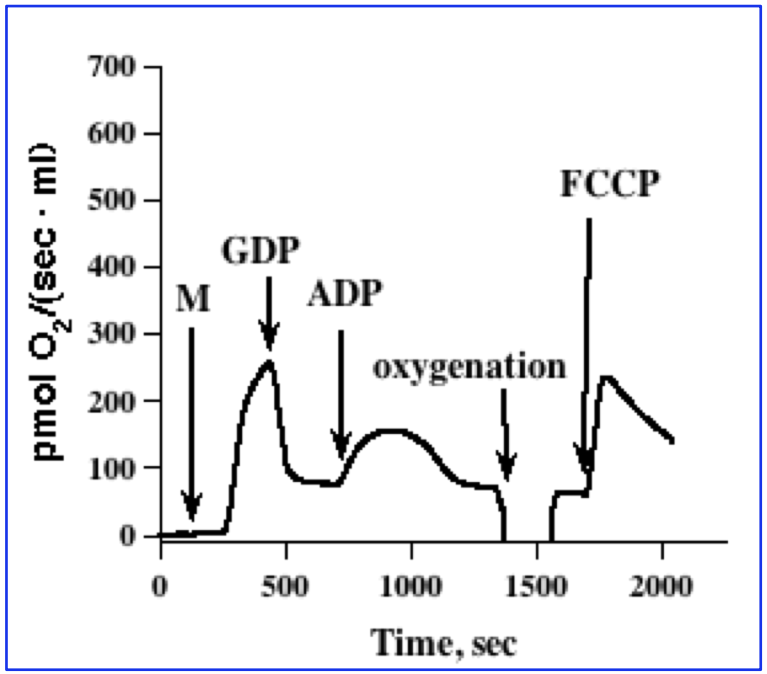
50 mM KCl
4 mM KPi
5 mM Hepes
1 mM EGTA
0.5% BSA

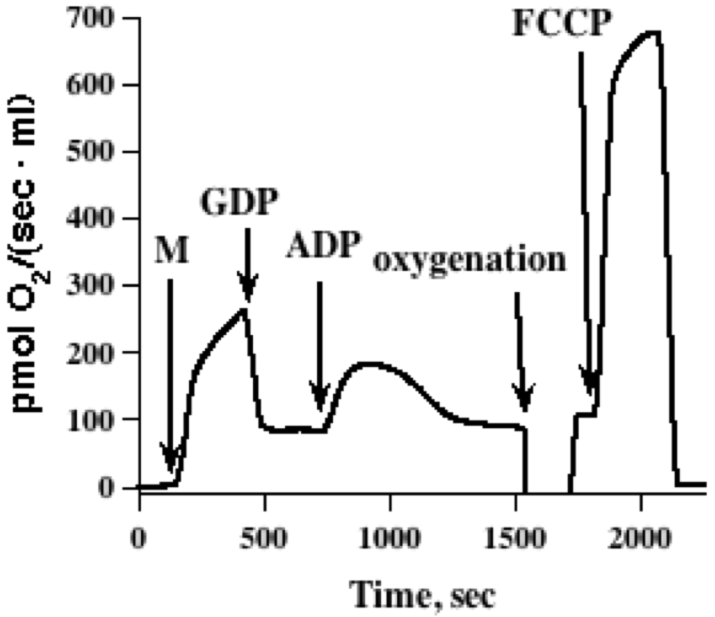
1 week at 4°C – slight browing of inguinal white adipose tissue

BAT/BRITE sucrose medium

125 mM sucrose
4 mM KPi
20 mM K+TES
1 mM EDTA
2 mM MgCl2
0.5% BSA







Kazak L, ……Spiegelman BM. Cell. 2015, 163(3):643-55.

In our hands

Our optimal medium