MitoFit Preprint Arch (2019) MiP/MitoEAGLE School Coimbra 2019
2019-07-04 doi:10.26124/mitofit:ea19.MiPSchool.0009

Inter-laboratory harmonization of respiratory protocols in permeabilized human muscle fibers

Doerrier Carolina[1], Gama-Perez P[2], Distefano G[3], Pesta D[4,5], Soendergaard SD[6], Chroeis KM[6], Gonzalez-Franquesa A[7], Goodpaster BH[3], Coen P[3], Larsen S[6], Gnaiger E[1,8], Garcia-Roves PM[2]

[1]*Oroboros Instruments, Innsbruck, Austria;* [2]*Dept Physiological Sciences, Univ Barcelona and Bellvitge Biomedical Research Inst, Spain;* [3]*Translational Research Inst Metabolism Diabetes, Florida Hospital, Orlando, FL, USA;* [4]*Inst Clinical Diabetology, German Diabetes Center, Leibniz Center Diabetes Research Heinrich-Heine Univ Düsseldorf;* [5]*German Center Diabetes Research, Munich, Neuherberg; Germany;* [6]*Dept Biomedical Sciences, Center Healthy Aging, Fac Health Sciences, Denmark;* [7]*The Novo Nordisk Center Basic Metabolic Research, Section Integrative Physiology; Univ Copenhagen, Denmark;* [8]*Dept Visceral, Transplant Thoracic Surgery, Daniel Swarovski Research Lab, Medical Univ Innsbruck, Austria.*
✉ carolina.doerrier@oroboros.at

https://www.mitofit.org/index.php/Doerrier_2019_MitoFit_Preprint_Arch_EA

Editor MitoFit Preprint Archives: Gnaiger E

Introduction

Permeabilized muscle fibers are extensively used for analysis of mitochondrial function in exercise and pathophysiological studies. Inter- and intra-laboratory comparisons of published results on permeabilized muscle fibers are difficult due to application of different experimental procedures, including sample preparation, substrate-uncoupler-inhibitor titrations (SUIT), respiratory media, and oxygen regimes. Oxygen dependence of mitochondrial respiration in permeabilized fibers (about 100-fold higher p_{50} compared to small living cells and isolated mitochondria [1]) reveals the requirement of using hyperoxic incubation conditions to avoid oxygen limitation of respiratory capacity [2]. However, controversial results on the oxygen dependence of permeabilized muscle fibers have been reported by different research groups using different respiration media in the presence or absence of the myosin II-specific inhibitor blebbistatin [3,4].

In the framework of COST Action MitoEAGLE, our main goals for the current study of permeabilized human muscle fibers are: (*1*) a comparison of protocols used in different research laboratories, (*2*) harmonization of results to address the reproducibility crisis [5], (*3*) evaluation of optimum experimental conditions, and (*4*) analysis of the causes of experimental variability.

Material and methods

We performed a blinded test with human permeabilized skeletal fibers. Six groups from Austria, Denmark, Germany, Spain, and USA measured simultaneously in the same laboratory mitochondrial respiration using high-resolution respirometry (O2k; Oroboros Instruments, Austria) in three human biopsies (*vastus lateralis*) from the same healthy volunteer sampled on three consecutive days. A total of 96 (32/day) permeabilized fiber preparations were assayed. The wet mass of permeabilized fibers ranged from 0.38 to 2.83 mg per chamber. Protocols were compared at several levels: (*1*) permeabilized fiber preparation; (*2*) respiration media MiR05-Kit and Buffer Z in the presence/absence of blebbistatin (25 µM), covering the most frequently used experimental conditions in the literature; (*3*) 'normoxia' (200-100 µM) versus hyperoxia (450-250 µM). The SUIT-008 protocol [6] was applied in all assays. Results were excluded from analysis if the cytochrome *c* flux control factor, FCF_c = ($I_{O2,cPM}$-$I_{O2,PM}$)/$I_{O2,cPM}$, exceeded 0.1 in the OXPHOS-state (Fig. 1; steps 2D and 2c). For abbreviations see Figure 1 and Gnaiger et al 2019 [7].

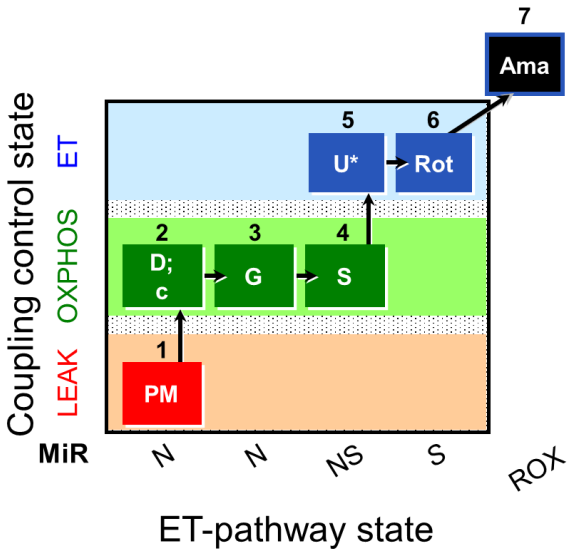

Figure 1. Substrate-uncoupler-inhibitor titration protocol (SUIT-008 O2 pfi D014). Sequential titrations and respiratory states. **1PM**: NADH-pathway (N-pathway) in the presence of 5 mM pyruvate and 2 mM malate in the N-LEAK state. **2D**: saturating ADP (N-OXPHOS state). **2c**: 10 µM cytochrome *c* for evaluating the integrity of the outer mitochondrial membrane. **3G**: 10 mM glutamate as an additional NADH-linked substrate (N-OXPHOS state). **4S**: 10 mM succinate (NS-OXPHOS capacity). **5U**: uncoupler titrations to evaluate the electron transfer- (ET-) capacity (NS-ET capacity). **6Rot**: inhibition of CI by rotenone (S-ET capacity). **7Ama**: inhibition of CIII by antimycin A (residual oxygen consumption, *Rox*).

Results and conclusions

NS-OXPHOS capacity was oxygen-limited under 'normoxic' compared to hyperoxic conditions in both media (Figure 2A-D). Blebbistatin did not prevent the decrease of respiration in the 'normoxic' regime (Figure 2A and 2C), and exerted minor effects on oxygen flux in both media (Figure 2E-F). These results indicate that oxygen dependence is critical and independent of experimental buffers and blebbistatin (Figure 2A-D). Comparing respiratory capacity in both media under hyperoxic conditions, oxygen flux per mass was higher in MiR05-Kit than in Buffer Z (Figure 2E-F). Evaluation of these trends will be completed based on an in-depth statistical analysis. Our inter-laboratory study provides a basis to harmonize published results on permeabilized human skeletal muscle fibers and establishes guidelines for selecting optimum experimental conditions.

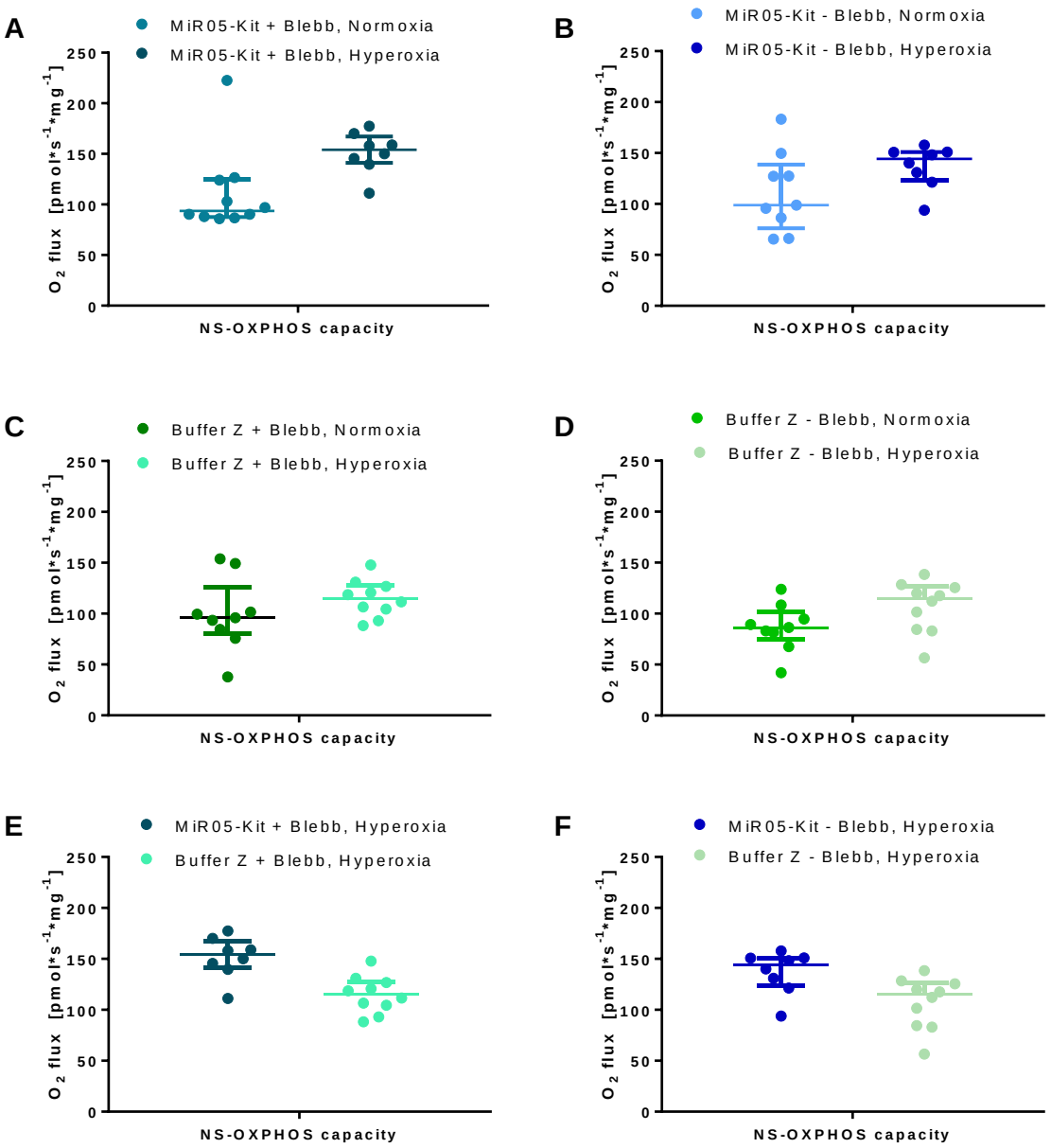

Figure 2. The effect of oxygen concentration and blebbistatin on mitochondrial respiration of permeabilized human skeletal muscle fibers in MiR05-Kit (A, B) and Buffer Z (C, D). Mass-specific NS-OXPHOS capacity (based on wet mass) supported by pyruvate, malate, glutamate and succinate. **(E, F)** Comparison of the two media at hyperoxia in the presence and absence of blebbistatin. A biopsy was taken on three consecutive days from the same person. Scatter plots and median with interquartile range show results from individual chambers (*n* = 8 to 10) with muscle fibers obtained from the three biopsies.

This preprint is based upon work from COST Action CA15203 MitoEAGLE, supported by COST (European Cooperation in Science and Technology)

Funded by the Horizon 2020 Framework Programme of the European Union

CD, EG: Supported by project NextGen-O2k, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.

References

1. Scandurra FM, Gnaiger E (2010) Cell respiration under hypoxia: facts and artefacts in mitochondrial oxygen kinetics. Adv Exp Med Biol 662:7-25.
2. Gnaiger E (2003) Oxygen conformance of cellular respiration. A perspective of mitochondrial physiology. Adv Exp Med Biol 543:39-55.
3. Perry CG, Kane DA, Lin CT, Kozy R, Cathey BL, Lark DS, Kane CL, Brophy PM, Gavin TP, Anderson EJ, Neufer PD (2011) Inhibiting myosin-ATPase reveals a dynamic range of mitochondrial respiratory control in skeletal muscle. Biochem J 437:215-22.
4. Bezuidenhout N, Doerrier C, Droescher S, Ojuka E, Gnaiger E (2016) Comparison of oxygen dependence of respiration in permeabilized mouse skeletal muscle fibers in two respiration media, MiR06Cr and Buffer Z containing Ctl, Cr and Blebbistatin. Abstract MitoFit Science Camp 2016.
5. Baker M (2016) 1,500 scientists lift the lid on reproducibility. Survey sheds light on the 'crisis' rocking research. Nature 533:452–4.
6. http://www.bioblast.at/index.php/SUIT-008_O2_pfi_D014
7. Gnaiger E, Aasander Frostner E, Abdul Karim N, Abumrad NA, Acuna-Castroviejo D, Adiele RC, et al (2019) Mitochondrial respiratory states and rates. MitoFit Preprint Arch doi:10.26124/mitofit:190001.v4.