



Isolated mitochondria or permeabilized tissues and cells

Erich Gnaiger

OROBOROS INSTRUMENTS Corp, high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
erich.gnaiger@oroboros.at; www.oroboros.at
Medical University of Innsbruck
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria



Summary: Whereas isolated mitochondria remain one of the gold-standards in studies of bioenergetics and mitochondrial physiology, permeabilized tissues and cells have become an alternative with several advantages. But some disadvantages have to be considered, too, for optimum experimental design and critical evaluation of results.

Section		Page
1.	Permeabilized tissue or cells versus isolated mitochondria (Ptic vs. Imt)	1
1.1.	General considerations.....	1
1.2.	Advantages of permeabilized tissue or cells (Ptic) ...	2
1.3.	Advantages of isolated mitochondria (Imt)	2
2.	Permeabilized tissue preparation and respirometry .	3
3.	References	4

1. Permeabilized tissue or cells versus isolated mitochondria in respirometry (Ptic vs. Imt): advantages and disadvantages

1.1. General considerations

1. Respiratory flux is frequently related to tissue wet weight or million cells in Ptic, and to mitochondrial protein in Imt. It is important to note that interpretation is very different of changes in respiratory flux per mass of tissue, million cells, or mitochondrial protein. For comparison of results, a common marker has to be quantified, such as citrate synthase activity ([MiPNet17.04](#)), Complex IV activity ([MiPNet08.12](#)), or cytochrome *aa*₃ content (Renner et al 2003).

2. Respiratory results on human skeletal muscle using Ptic (Gnaiger 2009) are in excellent agreement with data on Imt (Rasmussen et al 2001).
3. Substrates specific for feeding electrons into different complexes of the respiratory system, for various segments of the tricarboxylic acid cycle, and for transporters are used for functional evaluation of various sections of mitochondrial metabolism (Gnaiger 2012 MitoPathways: [MiPNet17.18](#)).
4. Substrate combinations are required for evaluation of maximum capacity of oxidative phosphorylation, to reconstitute physiological intracellular conditions ([Gnaiger 2009](#)).
5. Few studies with Ptic report the dependence of oxygen flux on various substrate concentrations, and direct comparison of Ptic and Imt is scarce or lacking (reviewed by [Gnaiger 2009](#)), except for ADP.
6. Optimum uncoupler concentrations for Ptic cannot be deduced from studies with Imt, since the sensitivities are different.

1.2. Advantages of permeabilized tissue or cells (Ptic)

1. Ptic needs less tissue or fewer cells than Imt. Using the OROBOROS Oxygraph-2k, only 1 mg wet weight of cardiac fibres is required per experimental test, in a 2 ml chamber at 37 °C, or 0.3 million cells (fibroblasts, endothelial cells).
2. Optimization of Imt may be significantly more time-consuming compared to the optimization of Ptic preparation. A set of standardized tests can be applied for quality control of Ptic or Imt preparation.
3. All types of mitochondria are experimentally accessible in Ptic, whereas Imt preparation allows for the separation of different mitochondrial populations (advantage), or it has been argued (but probably never shown experimentally) that Imt may involve the selective loss of damaged mitochondria.

1.3. Advantages of Isolated Mitochondria (Imt)

1. Imt preparation is required for separation and study of different mitochondrial subpopulations (Palmer et al 1977; Riva et al 2005).
2. The homogeneous suspension of Imt yields a representative average for the tissue sample, and fewer replica are required for averaging over heterogeneous subsamples of fibres.









3. The oxygen dependence of respiration in permeabilized muscle fibres is increased by two orders of magnitude, due to oxygen diffusion to the mitochondria in the small unperfused fibre bundle. Imt provide, therefore, the only choice for the study of mitochondrial oxygen kinetics (small isolated cells are a good model, as well). Low oxygen levels have to be strictly avoided in studies with muscle fibres, which is easily done, if one is aware of the problem (Gnaiger 2003; Pesta, Gnaiger 2012).
4. ADP has to be added to Ptic at high concentrations to achieve max. OXPHOS capacity, due to diffusion restriction and since the outer membrane may exert a barrier function different from Imt (Saks et al 2000; Gnaiger 2001).

2. Permeabilized Tissue Preparation and Respirometry

1. Evaluation of mechanical tissue separation: The degree of mechanical tissue separation may be evaluated by observing a change to a pale colouring of the separated fibre bundles (similar for liver). This is best observed when placing the Petri dish onto a dark background. Appropriate forceps have to be used. Initially, the main difficulties appear to be: (a) Application of too much tissue, which makes difficult the full attention to the mechanical separation of small amounts of tissue. A practical limit for routine experiments may be 10-20 mg wet weight of tissue, subsequently separated into 2-5 mg samples for experiments.
2. Mechanical and chemical permeabilization of the cell membrane: The mechanical tissue preparation leads to partial (skeletal muscle) or full permeabilization of the cell membrane (heart muscle; liver tissue). A preparation of fully intact cells, for the study of routine or endogenous respiration in the cell, cannot be obtained by this approach. Partially permeabilized preparations need additional chemical permeabilization (saponin or digitonin), by standardized incubation conditions which leave the outer and inner mitochondrial membranes intact. In merely mechanical permeabilized tissue, full permeabilization must be checked by addition of saponin or digitonin into the respirometer in the OXPHOS state (Pesta, Gnaiger 2012). Under these conditions, no stimulation of respiration is expected in fully permeabilized cells, whereas partial permeabilization is indicated by a stimulatory effect of added detergent (Gnaiger et al 1998).

3. Measurement of oxygen consumption of 1-2 mg of tissue per experimental run requires high-resolution respirometry. The instrumental limit of detection is 1 pmol O₂·s⁻¹·ml⁻¹.
4. Stability of tissue biopsies before the experiment is usually sufficient following standard procedures. Stability is prolonged by storing the biopsy in an intracellular preservation medium, since some cells are permeabilized during tissue sampling, minimizing storage after permeabilization, and by application of preservation medium ([MiPNet14.13](#)) after permeabilization and prolonged storage (Skladal et al 1994).
5. Stability of the tissue preparation in the respirometer depends on the application of a high-quality mitochondrial respiration medium (Gnaiger et al 2000). High stability allows for application of complex and extended substrate-uncoupler-inhibitor titration (SUIT) protocols (Gnaiger 2012).

3. References

- Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir Physiol* 128: 277-297. » 
- Gnaiger E (2003) Oxygen conformance of cellular respiration. A perspective of mitochondrial physiology. *Adv Exp Med Biol* 543: 39-56. » 
- Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int J Biochem Cell Biol* 41: 1837-1845. » 
- Gnaiger E (2012) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 3rd ed. *Mitochondr Physiol Network* 17.18. OROBOROS MiPNet Publications, Innsbruck: 64 pp. » 
- Gnaiger E, Kuznetsov AV, Lassnig B, Fuchs A, Reck M, Renner K, Stadlmann S, Rieger G, Margreiter R (1998) High-resolution respirometry. Optimum permeabilization of the cell membrane by digitonin. In *BioThermoKinetics in the Post Genomic Era* (Larsson C, Pålman I-L, Gustafsson L, eds) Chalmers Reproservice, Göteborg: 89-95. » 
- Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: *Life in the Cold*. (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: pp 431-442. » 
- Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Mark W, Steurer W, Saks V, Usson Y, Margreiter R, Gnaiger E (2004) Mitochondrial defects and heterogeneous cytochrome c release after cardiac cold ischemia and reperfusion. *Am J Physiol Heart Circ Physiol* 286: H1633-H1641. » 
- Palmer JW, Tandler B, Hoppel CL (1977) Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. *J Biol Chem* 252: 8731-8739.
- Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. *Methods Mol Biol* 810: 25-58. » 
- Rasmussen UF, Rasmussen HN, Krustrup P, Quistorff B, Saltin B, Bangsbo J (2001) Aerobic metabolism of human quadriceps muscle: in vivo data parallel measurements on isolated mitochondria. *Am J Physiol Endocrinol Metab* 280: E301-E307.

- Renner K, Amberger A, Konwalinka G, Kofler R, Gnaiger E (2003) Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. *Biochim Biophys Acta* 1642: 115-123. » 📄
- Riva A, Tandler B, Loffredo F, Vazquez E, Hoppel C (2005) Structural differences in twobiochemically defined populations of cardiac mitochondria. *Am J Physiol Heart Circ Physiol* 289: H868-H872.
- Saks VA, Kongas O, Vendelin M, Kay L (2000) Role of the creatine/phosphocreatine system in the regulation of mitochondrial respiration. *Acta Physiol Scand* 168: 635-641.
- Saks VA, Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, Tranqui L, Olivares J, Winkler K, Wiedemann F, Kunz WS (1998) Permeabilised cell and skinned fibre techniques in studies of mitochondrial function in vivo. *Mol Cell Biochem* 184: 81-100.
- Skladal D, Sperl W, Schranzhofer R, Krismer M, Gnaiger E, Margreiter R, Gellerich FN (1994) Preservation of mitochondrial functions in human skeletal muscle during storage in high energy preservation solution (HEPS). In: *What is Controlling Life?* (Gnaiger E, Gellerich FN, Wyss M, eds) *Modern Trends in BioThermoKinetics 3*, Innsbruck Univ Press: 268-271. » 📄

Protocols

[MiPNet08.12](#)

NO effect on mitochondrial oxygen kinetics at low oxygen.

[MiPNet17.04](#)

Citrate synthase – laboratory protocol.

[MiPNet14.13](#)

Mitochondrial respiration medium – MiR06

[MiPNet14.14](#)

Preparation of permeabilized muscle fibres

