**Analysis of Mitochondrial Respiratory Function in vitro, in vivo and in silico**

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**Introduction**

Impaired mitochondrial respiratory function (MRF, ATP synthesis capacity) has been documented in type 2 diabetes (T2D). Altered MRF in skeletal muscle is associated with changes at the molecular and cellular level. However, it is largely unknown how these different changes individually and collectively affect MRF in vivo. Both from a fundamental as well as a clinical point of view there is a clear need to improve our integrative understanding of this system. Hereeto we developed a new quantitative approach.

**Development in animal model**

Data collected from 8 and 25 week old Wistar rats (n=10), Figs. 1, 2.

1) **In vitro and molecular information**

![Depletion of in vitro PCr](image1.png)

Figure 1 mtDNA copy number (A) and protein content of electron transport chain (ETC) complexes determined by Western blotting (B). State 3, 4 and U respiration of isolated mitochondria by high-resolution respirometry (C).

2) **In vivo physiological information**

In vivo MRF was determined from the kinetics of phosphocreatine (PCr) recovery after exercise. PCr concentration dynamics were measured with in vivo $^{31}$P magnetic resonance spectroscopy.

![In vivo PCr](image2.png)

Figure 2 In vivo $^{31}$P MRS spectra (6.3 T) recorded at age 25 weeks (A). PCr rate constant ($k_{PCr}$) of animals at age 8 and 25 weeks (B). $^{31}$P MRS also provides information about the dynamics of in vivo pH during exercise. Error bars denote SEM.

3) **In silico**

The different data was integrated in a computational model (Fig. 3).

Monte Carlo simulations were performed to address the different sources of uncertainty in data and model.

Figure 3 Overview of the computational model. A) Cell model of skeletal muscle oxidative ATP metabolism, [5]. B) Tissue model. PCr dynamics are sensitive to cellular pH, which was included in the model.

Figs. 4-6 summarize results of the in silico analyses.

**Application in human (patho)physiology**

Healthy, normally active control subjects (age 31±12 mean±SD, n=6), subjects with sedentary lifestyle, athletes and T2D patients.

Figure 4 Predictions (black lines) of the relation between enzyme inhibition and state 3 respiration compared to experimental data (grey diamonds).

**Conclusion**

The added-value of applying in silico analyses for integration of in vivo and in vitro markers of mitochondrial function is demonstrated. A decrease in mitochondrial content was identified as the primary factor contributing to reduced MRF in vivo.

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**References**