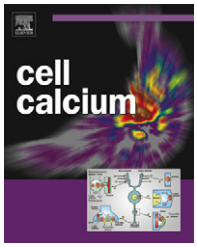
Cell Calcium (2008) 44, 123–133

journal homepage: www.elsevier.com/locate/ceca



REVIEW

# Mitochondrial $Ca^{2+}$ homeostasis in human NADH:ubiquinone oxidoreductase deficiency☆

**Peter H.G.M. Willems[a,*], Federica Valsecchi[a,b], Felix Distelmaier[a,b,c], Sjoerd Verkaart[a,b,1], Henk-Jan Visch[a,b], Jan A.M. Smeitink[b], Werner J.H. Koopman[a]**

[a] *Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*
[b] *Department of Paediatrics, Nijmegen Centre for Mitochondrial Disorders, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*
[c] *Department of General Pediatrics, Heinrich-Heine-University, Düsseldorf, Germany*

Received 16 October 2007; received in revised form 6 December 2007; accepted 4 January 2008
Available online 4 March 2008

**KEYWORDS**
Complex I deficiency;
Human skin fibroblasts;
Endoplasmic reticulum $Ca^{2+}$ content;
Mitochondrial $Ca^{2+}$ concentration;
Mitochondrial ATP production;
Cytosolic $Ca^{2+}$ removal;
CGP37157

**Summary** NADH:ubiquinone oxidoreductase or complex I is a large multisubunit assembly of the mitochondrial inner membrane that channels high-energy electrons from metabolic NADH into the electron transport chain (ETC). Its dysfunction is associated with a range of progressive neurological disorders, often characterized by a very early onset and short devastating course. To better understand the cytopathological mechanisms of these disorders, we use live cell luminometry and imaging microscopy of patient skin fibroblasts with mutations in nuclear-encoded subunits of the complex. Here, we present an overview of our recent work, showing that mitochondrial membrane potential, $Ca^{2+}$ handling and ATP production are to a variable extent impaired among a large cohort of patient fibroblast lines. From the results obtained, the picture emerges that a reduction in cellular complex I activity leads to a depolarization of the mitochondrial membrane potential, resulting in a decreased supply of mitochondrial ATP to the $Ca^{2+}$-ATPases of the intracellular stores and thus to a reduced $Ca^{2+}$ content of these stores.

☆ This work was supported by equipment grants of ZON (Netherlands Organization for Health Research and Development, #903-46-176), NWO (Netherlands Organization for Scientific Research, No: #911-02-008), by the European Union's sixth Framework Programme for Research, Priority 1 'Life sciences, genomics and biotechnology for health', contract number LSHM-CT-2004-503116, IOP Grant #IGE05003 of the Dutch Ministry of Economic Affairs, and a grant from the 'Forschungskommission der Medizinischen Fakultät, Heinrich-Heine -University, Düsseldorf'.

* Corresponding author at: 286 Biochemistry, Nijmegen Centre for Molecular Life Sciences (NCMLS), Radboud University Nijmegen Medical Centre, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands. Tel.: +31 24 3614589; fax: +31 24 3616413.
*E-mail address:* p.willems@ncmls.ru.nl (P.H.G.M. Willems).
[1] Current address: Department of Cell Physiology, Nijmegen Centre for Molecular Life Sciences (NCMLS), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

0143-4160/$ – see front matter 
doi:10.1016/j.ceca.2008.01.002

As a consequence, the increase in cytosolic $Ca^{2+}$ concentration evoked by a $Ca^{2+}$ mobilizing stimulus is decreased, leading to a reduction in mitochondrial $Ca^{2+}$ accumulation and ensuing ATP production and thus to a hampered energization of stimulus-induced cytosolic processes.


## The mitochondrial oxidative phosphorylation (OXPHOS) system

Mitochondria are double membrane-bound organelles that not only constitute the 'cellular power plants' but also are crucially involved in cell survival, apoptosis, redox control, calcium homeostasis and many metabolic and biosynthetic pathways [1,2]. To fulfil these diverse functions, mitochondria require a sufficiently negative membrane potential ($\Delta\psi$) across their inner membrane, which is maintained by the action of the electron transport chain (ETC). This chain is composed of four multisubunit assemblies that are embedded in the mitochondrial inner membrane: complex I (NADH:ubiquinone oxidoreductase; EC 1.6.5.3), complex II (succinate:ubiquinone oxidoreductase; EC 1.3.5.1), complex III (ubiquinol:cytochrome-*c* oxidoreductase; EC 1.10.2.2) and complex IV (cytochrome-*c* oxidase; EC1.9.3.1). Complexes I, III and IV actively translocate protons from the matrix into the intermembrane space using energy extracted from electrons passing through the chain. These electrons are liberated from NADH and $FADH_2$, at complexes I and II, respectively, where they are donated to the lipophilic electron carrier coenzyme Q for further transport to complex III. From there, electrons are shuttled to complex IV by cytochrome-*c*. At this complex, electrons are finally used for the reduction of oxygen to water. Together with complex V ($F_0/F_1$-ATP-synthase; EC 3.6.1.34), which uses the ETC-generated electrochemical proton gradient to produce ATP, the ETC complexes constitute the oxidative phosphorylation (OXPHOS) system. The OXPHOS system generates the vast majority of cellular ATP during oxidative metabolism and is composed of more than 80 different proteins, 13 of which are encoded by the mitochondrial DNA (mtDNA), and the remainder by the nuclear genome (nDNA; Ref. [3]). Seven of the mtDNA-encoded subunits are present in complex I, one in complex III, three in complex IV and two in complex V. Complex II consists solely of nDNA-encoded subunits. Disease-causing defects can occur in a single OXPHOS complex (isolated deficiency) or multiple complexes at the same time (combined deficiency).

Among the inborn errors of metabolism, mitochondrial disorders are the most frequent with an estimated incidence of at least 1 in 10,000 births (reviewed in Ref. [4]). The majority of these disorders are associated with defects in the OXPHOS system. These defects give rise to a variety of clinical manifestations, particularly in organs and tissues with high-energy demand such as brain (encephalopathies), heart (cardiomyopathies), skeletal muscle (myopathies) and liver (hepatopathies). One of the frequent OXPHOS disorders is Leigh Syndrome (OMIM 256000), an early-onset progressive neurodegenerative disorder, leading to death mostly within a few years after the onset of the symptoms. This disorder is characterized by lesions of necrosis and capillary proliferation in variable regions of the central nervous system. Clinical signs and symptoms comprise muscular hypotonia, developmental delay, abnormal eye movements, seizures, respiratory irregularities and failure to thrive. Other mitochondrial disorders caused by OXPHOS defects include mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS; OMIM 540000), myoclonic epilepsy with ragged red fibres (MERRF; OMIM 545000), neurogenic weakness, ataxia, retinitis pigmentosa/maternally inherited Leigh syndrome (NARP/MILS; OMIM 516060), Leber's hereditary optic neuropathy (LHON; OMIM 535000) and Mohr—Tranebjaerg syndrome (a.k.a. Deafness Dystonia Syndrome; OMIM 304700). More recently, OXPHOS dysfunction has also been implicated in diabetes, age-related neurodegenerative diseases, such as Parkinson's and Huntington's disease, and various forms of cancer [3,5—8].

## NADH:ubiquinone oxidoreductase or complex I of the OXPHOS system

Complex I is the largest complex of the OXPHOS system. Structurally, it consists of 45 subunits, 7 of which are encoded by mtDNA and the remainder by nDNA (Fig. 1; [9]). Its catalytic core comprises 14 evolutionary conserved core subunits [10], which, in humans, are encoded by the nuclear *NDUFV1*, *NDUFV2*, *NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS7* and *NDUFS8* genes and the mitochondrial *ND1—ND6* and *ND4L* genes. Complex I deficiency (OMIM 252010) was first described 25 years ago [11]. It is the major cause of mitochondrial disorders and disease-causing mutations have been found in both mtDNA- and nDNA-encoded subunits [4]. Because each cell contains many mtDNA copies (polyplasmy), not all of which may contain the disease-causing mutation (heteroplasmy), we restrict our research to nDNA mutations. Thus far, such mutations have been identified in all seven core subunits [12—18], in the so-called accessory or supernumerary subunits NDUFS4 [19], NDUFS6 [20] and NDUFA1 [21], in the complex I assembly factors B17.2L [22] and CIA30 (encoded by the *NDUFAF1* gene; [23]), and in the mitochondrial elongation factor G1 [24]. Considering the possible involvement of secondary factors in the cellular pathology of the deficiency, we study these mutations in the context of the genetic background of the patients. This approach might help to understand the large clinical heterogeneity in this subgroup of mitochondrial disorders [25].

The majority of complex I-deficient patients dies at a very young age. As far as our patient cohort is concerned, two children died in the neonatal period, whereas another six did not reach the age of one. Because there is hardly any muscle biopsy material available for research purposes, we use primary skin fibroblasts, which are more readily accessible. All patient fibroblast lines used in our studies are from children, in whom an isolated complex I deficiency has been confirmed in both muscle tissue and cultured skin