

27.1 Introduction

The general and main function of mitochondria is to generate energy for the living cell. The most important substrates for energy generation are pyruvate mainly produced from glucose, and fatty acids. Specific defects in the fatty acid oxidation cascade (i.e. various acyl-CoA dehydrogenase deficiencies, carnitine deficiency) are discussed in Chap. 14. Mitochondrial enzymes, which catalyse pyruvate converting reactions other than pyruvate oxidation, i.e. pyruvate carboxylase, are discussed in Chap. 15. This chapter is focused on deficiencies in the pyruvate oxidation pathway.

Under aerobic conditions pyruvate is transported into the mitochondrion, after which it is converted into acetyl-CoA by the enzyme complex pyruvate dehydrogenase. Acetyl-CoA is also the product of fatty acid β -oxidation. Acetyl-CoA can enter the citric acid cycle where electrons accumulated in carbon compounds are transferred to the electron carriers nicotinamide adenine dinucleotide (NAD^+) and flavin adenine nucleotide (FAD). The reduced coenzymes, NADH and FADH_2 , are the substrates for the subsequent process of oxidative phosphorylation by the respiratory chain (complexes I–IV) and complex V. The electrons are funnelled into the respiratory chain at the level of complex I in the case of NADH or complex II in the case of FADH_2 . The transfer of two electrons from NADH to the lipid-soluble redox carrier coenzyme Q is mediated by complex I. Complex III mediates the subsequent transfer of the electron pair from reduced coenzyme Q to the electron carrier cytochrome *c*. In the final step, cytochrome *c* is re-oxidized by complex IV, reducing molecular oxygen to water. The energy released during these electron transfer reactions is conserved in the form of an electrochemical proton gradient by means of a vectorial transport of protons across the mitochondrial inner membrane. Due to this proton transport a transmembrane potential is built up, which is used by complex V for the synthesis of the high-energy compound ATP from ADP plus inorganic phosphate.

Only if the overall capacity of the respiratory chain is sufficient can NADH and FADH_2 be oxidized adequately. If not the pyruvate dehydrogenase complex activity is inhibited by the accumulated NADH. Thus, defects

in the respiratory chain or other causes of an increase in the intramitochondrial NADH/NAD⁺ ratio lead to a decreased pyruvate oxidation rate *in vivo*, elevated pyruvate and lactate concentrations in body fluids [1, 2].

An increased intramitochondrial NADH concentration leads to an increase in cytosolic NADH through shuttles in the mitochondrial inner membrane, i.e. the malate-aspartate shuttle. The raised NADH/NAD⁺ ratio in the cytoplasm shifts the lactate dehydrogenase equilibrium in the direction of lactate. An increased lactate/pyruvate ratio can be found in most but not in all patients with a mitochondrial disorder. In the case of a disturbed respiratory chain in liver mitochondria, the reduced intramitochondrial redox state leads to an increased 3-OH-butyrate/acetoacetate ratio.

If one or more of the steps involved in the oxidation of pyruvate are dysfunctional, the amount of generated ATP is insufficient. Deficits of this energy-rich compound may lead to malfunctioning of the cell.

Unlike other cellular organelles, mitochondria have their own DNA. The human mitochondrial DNA (mtDNA), maternally transmitted, encodes 13 subunits of the respiratory chain enzyme complexes I, III, IV and complex V. In addition to structural genes, mtDNA also codes for 22 transfer RNAs and 2 ribosomal RNAs. This makes the oxidative phosphorylation system, which includes the respiratory chain (complexes I–IV) and complex V, unique as the different components are encoded by nuclear DNA (Mendelian inheritance) and mtDNA (maternal inheritance) with the exception of complex II, which is entirely nuclear encoded.

Clinically, mitochondrial disorders are a heterogeneous group of disorders that can affect various systems, in a mono- or multisystem pattern. Central nervous system, skeletal muscle and heart muscle are often involved. Moreover the same biochemical defect may cause diverse clinical phenotypes and, conversely, symptoms may be similar in patients with different biochemical defects. Patients may become symptomatic at any age and may show variable symptoms and outcome. Genetically, mitochondrial disorders can be due to mutations of either the nuclear or the mitochondrial genome, which will be transmitted by Mendelian or maternal inheritance, respectively. The mutation can also be sporadic. Despite this heterogeneity, several clinical presentations can be recognized such as Leigh disease, Leber's hereditary optic neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS), myoclonic epilepsy and ragged red fibres (MERRF), Kearns-Sayre syndrome (progressive external ophthalmoplegia plus retinitis pigmentosa and/or heart block, cerebellar syndrome and high CSF protein content), Pearson's syndrome (refractory sideroblastic anemia and exocrine pancreas dysfunction) and others. It must be stressed that clinical recognition of paediatric patients suffering from a mitochondrial disorder is rarely possible. There are nevertheless several signs and symptoms in children, which can be due to a mitochondrial disorder and indicate metabolic screening.

27.2 Nomenclature

Mitochondrial encephalopathies can be categorized on the basis of clinical, biochemical and molecular genetic criteria [3, 4]. In a substantial number of patients not a single but multiple enzyme defects are found.

The situation is further complicated by the fact that, despite in depth investigations, in a substantial number of patients the overall mitochondrial energy metabolism appears to be disturbed without any detectable enzyme or protein defect. Thus in about 35% of the muscle specimens with decreased pyruvate oxidation rates in vitro, no specific enzyme defect can be identified during routine investigations in our centre.

The primary genetic cause of deficiencies of the oxidative phosphorylation (OXPHOS) system may either be at the mitochondrial DNA (mtDNA) or at the nuclear DNA (nDNA) [5]. In general, mutations of mtDNA can be divided into major rearrangements and point mutations [6, 7].

MtDNA abnormality		Clinical phenotype
Deletion		Diabetes, Fanconi syndrome Cerebellar ataxia, hypogonadotropic hypogonadism, choroidal dystrophy
Point mutation		
A3243G	TRNA ^{leu}	MELAS
T3250C	TRNA ^{leu}	Isolated myopathy
A3251G	TRNA ^{leu}	Myopathy with lactic acidosis
T3271C	TRNA ^{leu}	MELAS
C3303T	TRNA ^{leu}	Isolated myopathy
T3394C	ND1	Long QT syndrome
G3460A	ND1	LHON
A8344G	tRNA ^{lys}	MERRF
G8363A	tRNA ^{lys}	MERRF
G11778A	ND4	LHON
G14459A	ND6	Leigh syndrome
T14484	ND6	LHON

In recent years much progress has been made in the characterization and mutational analysis of nuclear OXPHOS-genes. For detailed information the reader is referred to recent reviews concerning this topic [5, 8, 9].

The table below summarizes the presently known mutations in nuclear OXPHOS-genes.

Nuclear genetic classification of OXPHOS-disorders**A. Structural OXPHOS gene defects**

- Complex I deficiencies (Leigh and Leigh-like syndrome, mutations in the *NDUFS4*, -7 and -8 gene; hypertrophic cardiomyopathy and encephalomyopathy, mutations in the *NDUFS2* gene; macrocephaly, leucodystrophy and myoclonic epilepsy, mutations in the *NDUFV1* gene)
- Complex II deficiencies (Leigh and Leigh-like syndrome, mutations in the *Fp* gene)

B. Non-structural OXPHOS gene defects**1. Nuclear DNA-mitochondrial DNA defects**

- Reduced OXPHOS-enzyme activities (autosomal dominant progressive external ophthalmoplegia; mutations in the *ANT1* gene)
- Partial isolated complex IV as well as combined OXPHOS-complex deficiencies (MNGIE syndrome, mutations in the Thymidine phosphorylase gene)

2. Assembly defects

- Complex IV deficiencies (Leigh syndrome, mutations in the *Surf-1* gene; cardioencephalomyopathy, mutations in the *SCO2* gene; neonatal-onset hepatic failure and encephalopathy, mutations in the *SCO1* gene; Leigh and de Toni-Fanconi-Debré syndrome, mutations in the *COX10* gene)

OXPHOS, oxidative phosphorylation; NDUF, nuclear-encoded subunits of human complex I; Fp, flavoprotein; ANT, adenine nucleotide translocase; SCO, synthesis of cytochrome *c* oxidase (assembly gene); COX, cytochrome *c* oxidase.

Nomenclature

No.	Deficiency	Alternative name	McKusick number
Pyruvate dehydrogenase complex			
27.1	E _{1α} component of pyruvate DH complex	Pyruvate dehydrogenase	312170
27.2	E _{1α+β} component of pyruvate DH complex	Pyruvate dehydrogenase	179060
27.3	E ₂ component of pyruvate DH complex	Dihydrolipoyl transacetylase	245348
27.4	E ₃ component of pyruvate DH complex	Lipoamide dehydrogenase	246900
27.5	X component of pyruvate DH complex		245349
27.6	Pyruvate DH complex, unspecified		
27.7	Pyruvate DH phosphatase		
	Citric acid cycle		
27.8	Aconitase ^a		255125
27.9	E ₃ component of 2-oxoglutarate DH complex	Lipoamide dehydrogenase	246900
27.10	2-Oxoglutarate DH complex, unspecified	α -Ketoglutarate DH complex	203740
27.11	Succinate dehydrogenase	Complex II	252011
27.12	Fumarase	Fumarate hydratase	136850
	Respiratory chain		
27.13	Complex I	NADH dehydrogenase	252010/516000–516006
27.11	Complex II	Succinate dehydrogenase	252011
27.14	Coenzyme Q		
27.15	Complex III	Cytochrome <i>bc</i> ₁ complex	123980/ 124000/
27.16	Complex IV	Cytochrome <i>c</i> oxidase	220110/ 516030/
	Combinations of defects		
Energy converting system			
27.17	Complex V	ATP synthase, ATPase	
27.18	Coupling state		238800
	Transporting systems		
27.19	ATP/ADP translocator	ANT	103220
27.20	Malate/aspartate shuttle		254960
27.21	Protein import		251945
27.22	VDAC	Porin	

^a In association with a succinate dehydrogenase deficiency.
DH, dehydrogenase; VDAC, voltage dependent anion channel.

Extensive details concerning most listed OXPHOS defects may be found in recent reviews [2–5, 8, 10].

Classification of mitochondrial disorders using McKusick numbers is difficult, due to the complexity of the defects. McKusick numbers for diseases defined by a mtDNA mutation have recently been adapted (see nomenclature table).

Very recently defined OXPHOS disorders include a defect in the malate-aspartate shuttle (Hayes et al. [11]), a patient with a disturbed protein import (Schapira et al. [12]), an ATP/ADP translocator deficiency (Bakker et al. [13]), and VDAC deficiency (Huizing et al. [14]).

27.3 Metabolic Pathway

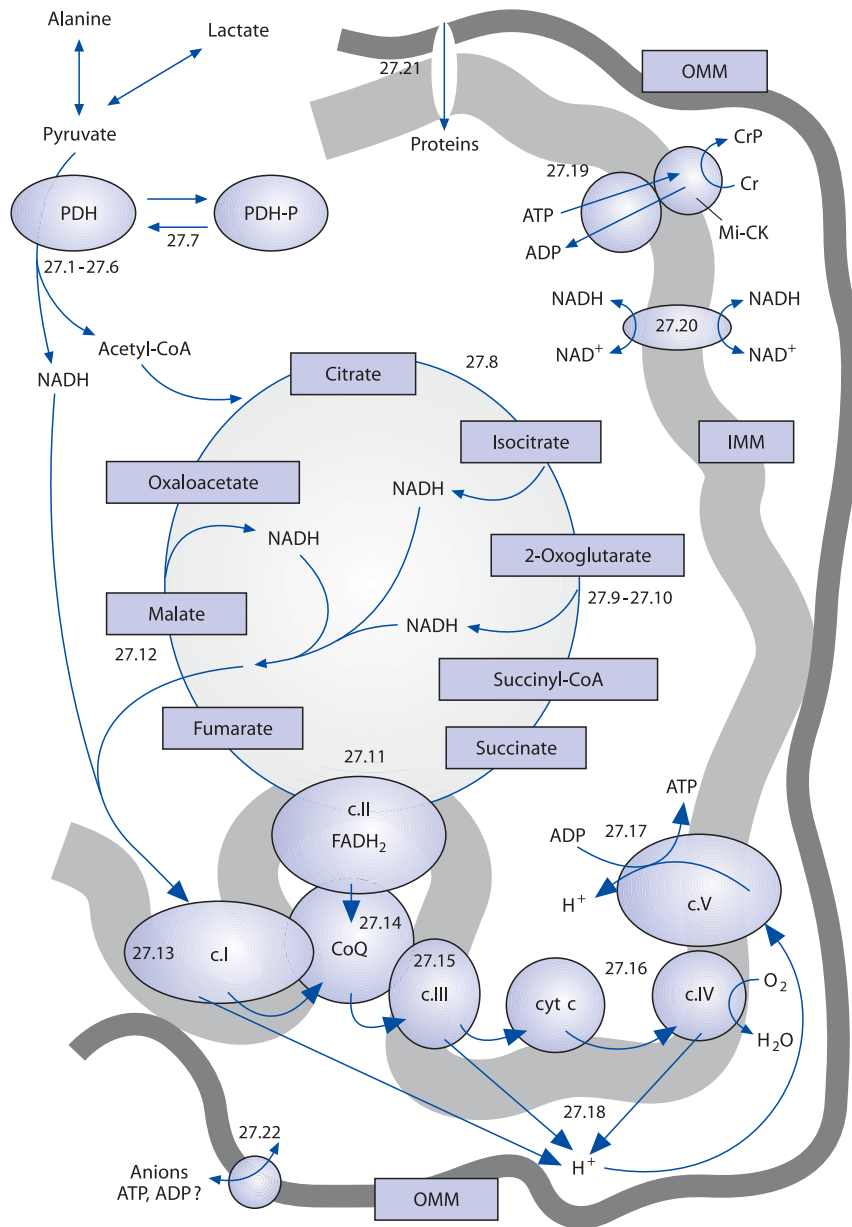


Fig. 27.1. Scheme of the mitochondrial energy metabolism (in muscle tissue). The described deficiencies are indicated by *numbers*, which refer to Table 27.1. *PDH(-P)*, (phosphorylated) pyruvate dehydrogenase; *c.I*, *c.II*, *c.III*, *c.IV*, *c.V*, complexes I, II, III, IV and V, respectively, of the respiratory chain; *CoQ*, coenzyme Q; *cyt c*, cytochrome c; *Cr(P)*, creatine (phosphate); *IMM*, inner mitochondrial membrane; *OMM*, outer mitochondrial membrane

27.4 Signs and Symptoms

Both the clinical and clinical-chemical abnormalities are very heterogeneous and often aspecific in patients suffering from a mitochondrial disorder [1, 10, 15, 16]. The symptomatology varies in age at onset (from birth to adulthood) and course (rapidly progressive, static). In some patients only one tissue seems to be affected, while other patients seem to suffer from a multisystem disorder.

In the majority of the patients muscular and/or neurological complaints are the main presenting symptoms. Some symptoms are age-dependent (i.e. failure to thrive at neonatal age and exercise intolerance in adulthood), while others (i.e. hypotonia, retardation) can present at any age.

Name	Symptoms	McKusick number	Abnormality in mtDNA ^a
PEO	Progressive external ophthalmoplegia	550000	Large deletion
KSS	Ophthalmoplegia, heart block, retinopathy	530000	Large deletion
Leigh syndrome	Subacute necrotizing encephalomyelopathy	516060	T8993C, T8993G
LHON	Leber's hereditary optic neuroretinopathy	516003	G11778A and others
MELAS	Mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes	540000	A3243G, T3271C
MERRF	Myoclonic epilepsy, ragged-red fibers	545000	A8344G, T8356C
MNGIE	Mitochondrial neuropathy, gastrointestinal disorders, encephalopathy	550900	Multiple deletions ^b
NARP	Neuropathy, ataxia, retinitis pigmentosa	551500	T8993G
Pearson syndrome	anaemia, pancreas dysfunction	557000	Large deletions

^a Only the most frequently observed mutations are given.

^b Caused by mutations in the nuclear encoded thymidine phosphorylase gene (confusing-point mutations or multiple deletions?).

Many patients, particularly children, who meet the morphological, biochemical and/or molecular biological criteria for a mitochondrial disorder, can not be classified into one of the aforementioned entities. An additional complication is that in a few patients the clinical picture gradually changes from one well defined clinical phenotype into another one.

The most frequently found clinical symptoms are [17]:

CNS	Seizures
	Hypotonia/hypertonia
	Spasticity
	Transient paraparesis
	Lethargy/coma
	Psychomotor retardation/regression
	Extrapyramidal signs
	Ataxia (episodic)
	Dyspraxia
	Central hypoventilation
	Deceleration/acceleration of head growth
	Blindness (cortical)
	Deafness (perceptive)
Skeletal muscle	Exercise intolerance/easy fatiguability
	Muscle weakness
Heart	Cardiomyopathy (hypertrophic or dilated)
	Conduction abnormalities
Eyes	Ptosis
	Restricted eye movements
	Strabismus
	Cataract
	Pigmentary retinopathy
	Optic atrophy
Liver	Hepatic failure
Kidney	Tubular dysfunction
Endocrine	Diabetes insipidus
	Delayed puberty
	Hypothyroidism
	Hypoparathyroidism
	Diabetes mellitus
	Exocrine pancreas dysfunction
	Primary ovarian dysfunction
	Diarrhoea (villous atrophy)
	Intestinal pseudo-obstruction
Gastrointestinal	
Other	Failure to thrive
	Short stature
	Pancytopenia
	Anemia

No single clinical feature is specific or distinctive. A patient is suspected to suffer from a mitochondrial disorder if demonstrating at least two chronic and unexplained symptoms from this extended list, preferably occurring in two unrelated organs [15, 16].

The following laboratory investigations are worthwhile for diagnosing mitochondrial disorders:

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- Lactate and pyruvate in blood
 - Ketone bodies in blood
 - Amino acids in blood and urine
 - Lactate and amino acids in CSF
 - Organic acids in urine
 - CT/MRI or MRS of brain
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Urine for amino acid and organic acid analysis should be collected in the fed state. If no lactate increase is found in body fluids, an oral glucose loading test should be performed (see Sect. 27.7).

Accumulation of compounds related to the mitochondrial pathway can be detected in one or more body fluids of most patients [1, 2, 15]. Special attention has to be paid to the lactate concentration. Excess of lactate and alanine will be produced after reduction or transamination of accumulated pyruvate (see Fig. 27.1). If there is a severe block in the pyruvate oxidation pathway, and the produced lactate can not adequately be removed by peripheral tissues, it accumulates in blood, urine and/or cerebrospinal fluid, dependent upon the affected tissue(s). A decreased activity of the respiratory chain will shift the equilibrium of the lactate dehydrogenase reaction to conversion of pyruvate to lactate (see also Sect. 1). Thus, patients with a respiratory chain defect should demonstrate an increased lactate/pyruvate ratio in blood, whereas pyruvate dehydrogenase deficiency should result in a normal lactate/pyruvate ratio. However, this tool for differential diagnosis is not helpful in all cases. Furthermore, some patients do not accumulate lactate in blood or urine.

The concentration and associated ratio of the ketone bodies, acetoacetate and 3-hydroxybutyrate, may also be helpful [15, 18, 19]. Ketosis and keto-aciduria are observed in certain patients with a mitochondrial disorder. A non-physiological increase of ketone bodies postprandially may be another indicator of a mitochondrial defect (Saudubray et al). Increased 3-hydroxybutyrate/acetoacetate ratio may suggest a defect in the respiratory chain in liver tissue.

Amino acid determination in blood and urine may be helpful. Alanine is increased in many patients with a mitochondrial disorder (see above). Deficiency of E₃ complex leads to branched-chain amino acid elevation. Severe generalised amino aciduria associated with DeToni-Fanconi-Debré tubulopathy may indicate a respiratory chain defect.

Urinary excretion of specific organic acids may suggest a mitochondrial defect. Cytochrome *c* oxidase defects and other respiratory chain defects have been demonstrated in some patients with ethylmalonic aciduria or 3-methylglutaconic aciduria. In certain cases intermediates of the citric acid cycle are also found in increased amounts in the urine.

Section 27.8 contains a flow chart that is useful in diagnosing these conditions (see Fig. 27.2).

Imaging techniques (CT and MRI) can reveal important information about the localization of lesions. Symmetric lesions in basal ganglia and brainstem are strongly suggestive of Leigh syndrome. It is possible to detect increased content of lactate in specific regions of the brain by proton MRS [17, 18].

27.5 Reference Values

Compound	Serum/blood ($\mu\text{mol/l}$)	Urine (mmol/mol creat)	Cerebrospinal fluid ($\mu\text{mol/l}$)
Lactate	450–1800 (B)	<270 (<2 months) <200 (2 months–2 yr) <85 (>2 yr)	1100–1700
Pyruvate	60–100 (B)		80–140
Lactate/pyruvate ratio	<15 (B)		<15
Alanine	200–500 (P, S)	70–250 (<6 months) 30–125 (6 months–7 yr) 20–70 (>7 yr)	16–41 (<1 yr) 13–31 (>3 yr)
Acetoacetate ^a	5–50 (B)		
3-Hydroxybutyrate ^a	15–90 (B)		
3-Hydroxybutyrate/ acetoacetate ratio ^a	<1.0 (B)		
Ammonia	10–50 (P)		
CK	<200 (M) (S, U/l) <170 (F)(S, U/l)		
Protein (total)			450–1100 (<1 month, mg/l) 160–650 (1 month– 50 yr; mg/l)
Ethylmalonic acid		<20	
3-Methylglutaconic acid		<20	

Reference values are dependent upon the method used. The listed values should be used only as a guide.

B, blood; S, serum; P, plasma; M, male; F, female; U/l, units/liter.

^a Nonfasting.

27.6 Pathological Values

Compound	Blood ($\mu\text{mol/l}$)	Urine (mmol/mol creat)	Cerebrospinal fluid ($\mu\text{mol/l}$)
Lactate	>2000 (B)	>350 (<2 months) >300 (2 months–2 years) >130 (>2 years)	>2000
Pyruvate	>130 (B)		>200
Lactate/pyruvate ratio	>17 (B)		>17
Alanine	>500 (P, S)	>300 (<6 months) >150 (6 months–7 years) >100 (>7 years)	
Acetoacetate + 3-OH-butyrate	Postprandial increase (B)		
Ammonia	>100 (P)		
CK	>200 (M) (S, U/l) >170 (F) (S, U/l)		
Protein (total)			>1300 (>1 month, mg/l)
Ethylmalonic acid		>25	
3-Methylglutaconic acid		>25	

B, Blood; S, Serum; P, Plasma; M, Male; F, Female.

An increased lactate concentration in CSF is an important indicator for CNS involvement. Increased lactate concentrations in blood are also indicative, but can easily be caused by stress (fear of venapuncture), excessive muscle contractions (status epilepticus), anoxia and other conditions. A reliable value is only obtained from two or more blood lactate determinations. Some patients with a proven mitochondrial defect do not show lactate accumulation in blood or urine, but CSF lactate is frequently increased. Urinary lactate excretion can be secondarily increased in some types of organic aciduria [2].

In healthy people the rate of ketogenesis, and therefore the concentration of acetoacetate and 3-hydroxybutyrate in the blood, will decrease after meals, but may increase in mitochondrial disorders [15, 20]. Increased serum ammonia, creatine kinase or CSF protein concentration is not indicative for a mitochondrial disturbance. If found, urea cycle defects, liver cirrhosis, muscle dystrophy or brain necrosis must be considered. Patients with Kearns-Sayre syndrome and Leigh syndrome, however, often have increased protein concentrations in the CSF.

27.7 Loading Tests

■ Glucose Loading Test

A standardized oral glucose loading test, with 2 g glucose/kg body weight, and blood sampling at 0, 30, 60, 90, 120 and 180 min after intake, provides insight into the capacity for in vivo pyruvate oxidation. Pyruvate oxidation may be impaired if the peak increase in blood lactate surpasses 1 mmol/l or double the value of the basal concentration. It is unclear whether the liver mitochondria are more involved in this test than the muscle mitochondria. Even normal test results do not totally exclude mitochondrial dysfunctioning.

Fasting tests are not informative.

■ Exercise Test

Phosphorus nuclear magnetic resonance (^{31}P NMR) is a very useful technique to demonstrate a possible mitochondrial disturbance in vivo [19].

Furthermore, ^{31}P NMR studies can be used to follow therapeutic interventions. As a rule, a fast decline in creatine phosphate content during exercise, no synthesis of phospho-monoesters, and a delayed creatine phosphate resynthesis after exercise are found in patients with a mitochondrial disorder.

27.8 Diagnostic Flow Chart

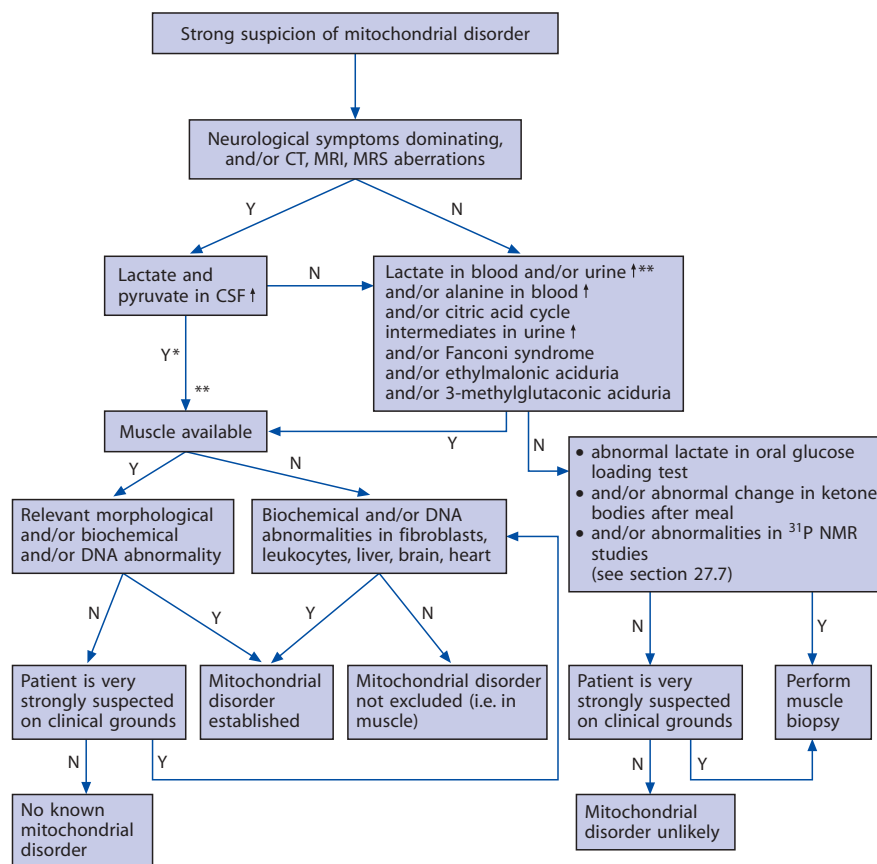


Fig. 27.2. Flow chart for diagnosing deficiencies in mitochondrial energy metabolism.

* Although not strictly necessary, it is worthwhile to establish the lactate concentration in blood and urine, the organic acids in urine and the amino acids in serum, urine and CSF (for follow-up, therapy, tissue specificity). ** Determine pyruvate carboxylase activity. CT, computed tomography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy

A broad spectrum of possible mitochondrial abnormalities, rather than a specific enzyme or DNA aberration, can be detected by following this scheme. Only a few patients present with symptoms indicative for a specific defect. Measurement of various enzyme activities is required for proper diagnosis.

The scheme forms a rough guide. A proper interpretation of the signs and symptoms is very important (Sect. 27.4).

27.9 Specimen Collection

In order to arrive at a definitive diagnosis, biochemical examination of tissue specimens is necessary. As a rule, the patient under investigation should not be on vitamin therapy. Therapy should be stopped, if possible for at least 1–3 weeks, before performing a muscle biopsy.

For most biochemical determinations one should ask the diagnostic centre for information about specific requirements as to the practice of collecting and transporting material. Especially in the case of enzyme analysis in tissues or cells, one must consult the diagnostic laboratory in advance about the conditions for removal, preparation, storage (usually at -70°C) and transport of the specimens. If fresh tissue is to be studied, a special, ice-cold buffer must be available. The specimen must be at the laboratory within 2 hours after removal of the tissue. In this material (with intact mitochondrial membranes) substrate oxidation rates and ATP production rates can be measured, as well as single enzyme activities. In frozen tissues only the latter tests can be measured.

The physician should inform the laboratory about the clinical findings to ensure an adequate analysis. It is important to discuss which type of tissue or cell is preferable in each individual case, thereby causing the patient as little inconvenience as possible. Tissue-specific expression of mitochondrial deficiencies renders fibroblasts and lymphocytes less universally appropriate than skeletal muscle.

In the case of unexpected death, blood and urine specimens should be collected immediately after death, and stored for possible additional studies. For enzymatic purposes tissues must be removed within 1–2 h after death and should be frozen immediately in liquid nitrogen. Skin biopsy can be performed as late as 48 h after death.

Because few reference values are generally available for neonates, it is recommended to perform a muscle biopsy after the first month of life, unless a life-threatening situation exists. mtDNA analysis can, in principle, be performed in all types of tissues or cells available. However, the extent of heteroplasmy, i.e. the percentage of mutated mtDNA related to the total amount of DNA, varies from tissue to tissue.

Test	Material	Storage	Pitfalls
Lactate	B, CSF, U	-20 °C	Prevent glycolysis
Pyruvate	B, CSF	-20 °C	Prevent glycolysis and LDH activity
Amino acids	S, P, U, CSF	-20 °C	-
Blood gases	B	No storage allowed	-
CK	S	-20 °C	-
Acetoacetate	B	-20 °C	Feeding state is important
3-Hydroxybutyrate	B	-20 °C	-
Carnitine	S, M, L, FB	-20 °C	-
Organic acids	U	-20 °C	-

Screening	Material	Storage	Pitfalls
Amino acids	U	-70 °C	Antiepileptic drugs and antibiotic artefacts
Biochemical activities			
Pyruvate dehydrogenase	M, FB, L, CV	-70 °C	
2-Oxoglutarate dehydrogenase	M, FB, L	-70 °C	
Fumarase	M, L, FB	-70 °C	
Respiratory chain enzymes	M, FB, L, CV	-70 °C	
ATPase	M, FB	-70 °C	
ATP/ADP-translocator	M, L, FB	-70 °C	
Substrate oxidations	MF, FB, LF	No storage allowed	Maintain at 0 °C
ATP production	MF, FB	No storage allowed	Maintain at 0 °C
Oxygen consumption	MF, LF, BrF	No storage allowed	Maintain at 0 °C
Coupling state	MF, LF	No storage allowed	Maintain at 0 °C
DNA			
Respiratory chain enzymes	M, B, FB	-20 °C	

M, muscle (fresh or frozen); L, liver (fresh or frozen); MF, fresh muscle required; LF, fresh liver required; BrF, brain (fresh); FB, fibroblasts; CV, chorionic villi.

27.10 Prenatal Diagnosis

At present prenatal diagnosis in mitochondrial disorders can be performed in families in which the proband is suffering (suffered) from a complex I, complex IV or pyruvate dehydrogenase complex deficiency, at least in our centre. A prerequisite for prenatal diagnosis at the enzyme level is the establishment of the defect in fibroblasts from the proband. Prenatal diagnosis is preferably performed in native chorionic villi because they can be obtained earlier in pregnancy as compared with amniocytes. Moreover, it is not necessary to cultivate chorionic villi in contrast with amniocytes, thus

reducing the time of the diagnostic procedure considerably. In case the investigation of chorionic villi yields no conclusive result, amniocytes can also be investigated. Mutations in mtDNA in the proband excludes a reliable performance of prenatal diagnosis due to the unpredictable percentage of heteroplasmy in the fetal cells. At the DNA level prenatal diagnosis can be performed in families in which the index patient has (had) a pyruvate dehydrogenase complex deficiency, mutation in the E1I gene, or in complex I/complex IV deficiency with a proven mutation in one of the known nuclear encoded genes. Mutation-based prenatal diagnosis is expected to increase in the future.

27.11 Initial Treatment

Symptomatic treatment covers a variety of conventional medical practises. Heavy exercise should be withheld, preventing lactic acidosis or other consequences. Anti-convulsive drugs are given in case of an encephalopathy complicated by convulsions. It is advisable to avoid drugs such as valproate, which depletes carnitine and alters respiratory chain activity. During an acute crisis, provoked by an intercurrent infection, exercise, prolonged fasting or occurring spontaneously, buffering blood pH and/or removing toxic substances by dialysis is indicated. Dietary alteration may be effective if the pathophysiology involves accumulation of toxic precursors whose major source is nutritional. Carnitine can be supplemented in patients in whom the carnitine synthesis is disturbed or the concentration is decreased for unknown reasons. Cofactor supplementation may be efficacious. Pathological amounts of oxygen radicals may form in mitochondrial disorder; Vitamin E supplementation may be beneficial.

Unfortunately in patients with a mitochondrial disorder all therapy is as yet palliative. Sporadic favourable responses justify therapeutic trials in every new patient [22].

27.12 Summary

Thousands of patients with mitochondrial abnormalities have been detected. Clinically, they have symptoms which cannot always be explained by lack of energy. About 20 different defects of enzyme systems, and more than 100 different mutations in mitochondrial and nuclear DNA, have been described. Certain enzyme and DNA abnormalities are nonspecific. Morphologic studies can demonstrate an abnormal number or localization of mitochondria, or ultrastructural aberrations, such as crystal inclusions.

Muscle mitochondrial abnormalities may be 1° or 2° to other causes.

The diagnostic route is not straightforward. Scrupulous clinical observation, intensive cooperation with technical specialists, and last, but not least, experience are required for an adequate diagnostic protocol.

During the coming decade, protein, cell biological and DNA studies will result in additional knowledge about the pathogenetic mechanisms causing different types of mitochondrial disorders, and the modes of inheritance. This will improve the possibilities for pre- and postnatal diagnosis, and hopefully also for treatment.

Acknowledgement. The authors are indebted to the Princess Beatrix Fonds, which supported our research on mitochondrial cytopathies during the past decade.

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