UK Metabolic Biochemistry Network Recommendations for the Investigation of Rhabdomyolysis for Inherited Metabolic Disorders

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Disclaimer: These are laboratory guidelines reflecting current best practice in specialist metabolic laboratories in the UK. They are not evidence based but reflect expert opinion. MetBioNet cannot accept any responsibility for use of these guidelines.

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Definition of rhabdomyolysis

There is no universally agreed clinical definition of rhabdomyolysis – literally dissolution of striped muscle. It is characterized by leakage of muscle cell contents, including creatine kinase and myoglobin, into the extracellular space and the circulation. Laboratory diagnosis is by measurement of an **acute** increase in serum concentration of creatine kinase (CK) to more than five times the upper normal limit of normal when myocardial infarction has been excluded as a cause.[1] This is accompanied by symptomatic muscle involvement including myalgia, weakness and/or swelling.

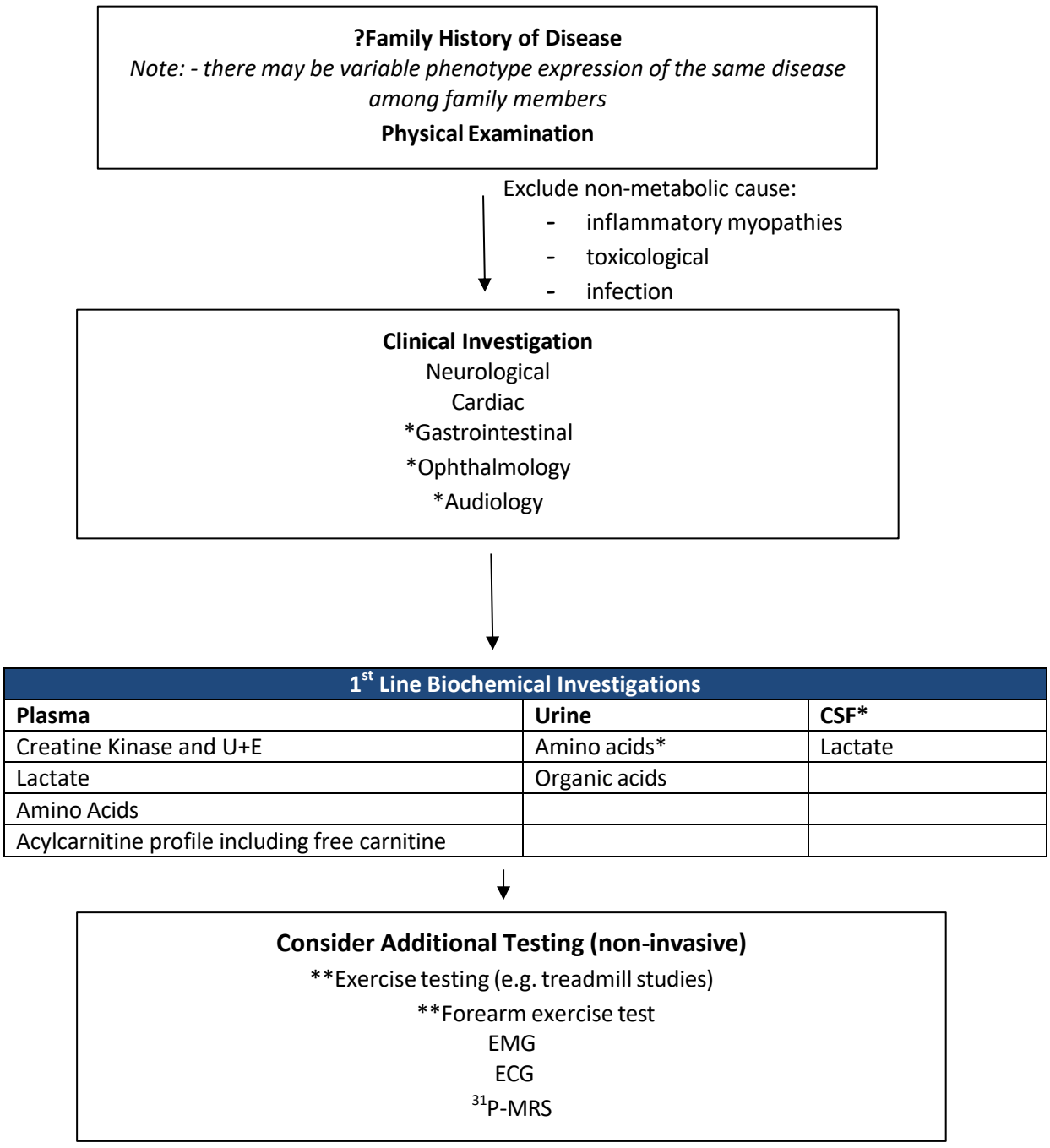
CK peaks in serum at 30-50 hours following muscle injury. Myoglobin peaks in serum much sooner than CK at around 17 hours post injury and visible myoglobinuria (tea or cola coloured urine) occurs when urinary myoglobin exceeds 300-1000 ng/ml (normal <10 ng/ml). However, the term myoglobinuria is insufficiently defined to be of use in the definition of rhabdomyolysis.[2] Nevertheless, patients should be asked about changes in urine coloration. Urine should be dipstick tested: Haemoglobin without erythrocytes indicates myoglobinuria.[3]

Guidelines

Patients with unexplained rhabdomyolysis (i.e. after exclusion of acquired causes -for extensive list see Beetham R 2000 [2]) should be investigated for possible metabolic causes. Below is a suggested outline for investigation of patients with no specific clues to the diagnosis (Fig. 1). This includes clinical assessment and first line blood and urine biochemical testing. Non-invasive non-biochemical testing may also be undertaken at this stage (appendix 1). It may be useful to use exercise as a tool to facilitate evaluation (appendix 2). Such tests will include cycle ergometers or treadmills that will provide information on cardiac output during exercise, oxygen extraction per unit of blood, ventilation relative to oxygen uptake and lactate production. The forearm non-ischaemic exercise test is potentially very useful when undertaken by experienced clinicians for evaluating the probability of some GSD's or myoadenylate deaminase deficiency in adolescents and adults (see protocol in appendix 2).[4] However, this test has been made somewhat obsolete since the ready availability of mutation analysis.

An in-depth description of metabolic myopathies is described elsewhere.[5,6] More specialist biochemical testing is only indicated on muscle or skin fibroblasts after this preliminary assessment and investigations have been completed and evaluated (Fig. 2). The specialised laboratory investigations are detailed in appendix 3.

Figure 1: Suggested outline for investigation of patients presenting with rhabdomyolysis

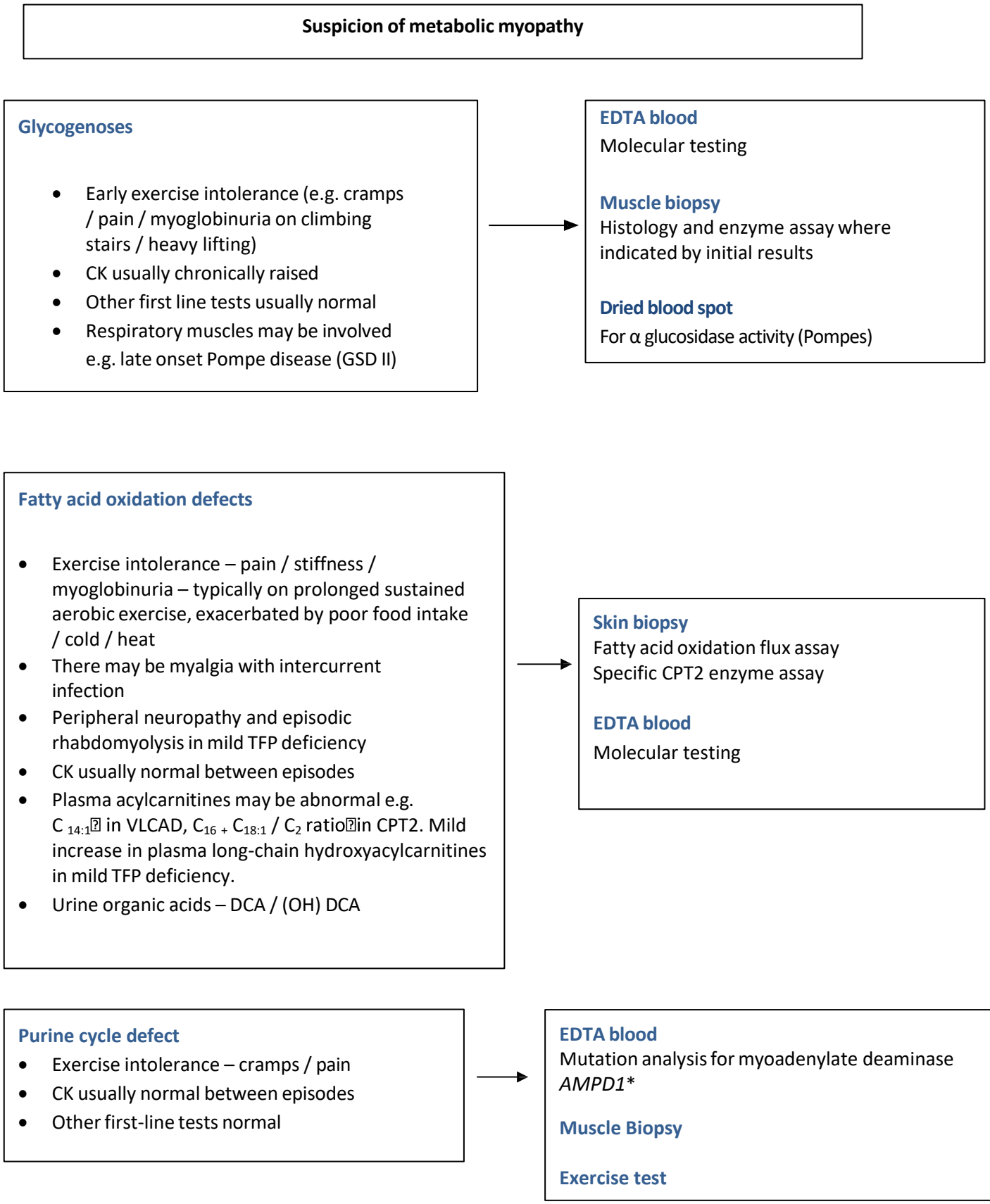


1st Line Biochemical Investigations		
Plasma	**Urine**	**CSF***
Creatine Kinase and U+E	Amino acids*	Lactate
Lactate	Organic acids	
Amino Acids		
Acylcarnitine profile including free carnitine		

*Particularly important in the investigation of possible respiratory chain disease

** For uses and protocol see appendix 2

Figure 2: Suspicion of metabolic myopathy



Respiratory Chain Defects

- Exercise intolerance, occasionally myoglobinuria
- Often more than one organ affected
- Normal or raised plasma lactate
- CSF lactate sometimes raised (may be normal)
- Normal or raised CK
- May have abnormal urine organic acids (increased urinary tricarboxylic acid cycle intermediates and/or dicarboxylic aciduria, 3-methylglutaconic aciduria)
- Normal or raised plasma alanine, reflecting lactic acidaemia
- May have generalised amino aciduria

→

Muscle biopsy

Respiratory chain complexes May also include ubiquinone

Histology

May have ragged red/ blue fibres If isolated complex III deficiency on muscle biopsy – test mutations in the cytochrome b mtDNA gene

Lipin 1 deficiency

- Rhabdomyolysis can occur after exercise, fever, anaesthesia or fasting
- Accounts for approx. 10% of patients with severe recurrent childhood rhabdomyolysis
- Cardiac arrest, sometimes with hyperkalaemia, is reported
- Normal acylcarnitines

→

Molecular testing

LPIN1 gene

RYR1 mutations

- At risk of malignant hyperthermia, including rhabdomyolysis, after exposure to certain

→

Molecular testing

RYR1 gene

CPT2 – Carnitine palmitoyltransferase type 2, VLCAD – very-long chain acyl-CoA dehydrogenase deficiency, RYR – Ryanodine receptor 1

* Where a homozygous mutation in the myoadenylate deaminase gene is confirmed, further studies should continue to establish whether or not a second defect is present, as most patients with isolated myoadenylate deaminase deficiency are asymptomatic and the possibility of another contributary defect should therefore be considered. MADA mutations are common in the population (1-2%) but the majority are asymptomatic due to alternate splicing of the gene. [7,8]

Advice should be sought from your local Molecular Genetics Laboratory as to the correct gene panel to request. Panels exist for 'acute rhabdomyolysis', 'congenital myopathy' and 'likely inborn error of metabolism'.

Appendix 1

Specialised non-invasive non-biochemical testing

Specialised non-invasive testing as outlined below is not universally available in the UK but may, in certain specialist centres, provide additional information that can be used to indicate the nature of the underlying defect e.g. respiratory chain defect. It can be particularly useful to undertake this testing after exercising (see appendix 2).

Electromyography (EMG) may show myopathic features such as fibrillations, positive sharp waves, and myotonic discharges. Electrocardiography (ECG) shows left ventricular or biventricular hypertrophy in some patients with myopathy, whereas radiographic evidence for cardiomegaly is uncommon.

Phosphorus – magnetic resonance imaging (P-MRS) can be particularly useful in myopathies associated with impaired energy metabolism. MR spectroscopy is used non- invasively for the direct and continuous assessment of tissue metabolites and is particularly useful in repeat monitoring of muscle bioenergetics during rest, exercise and recovery. Not all compounds that contain phosphorus produce visible signals on MRS. In human muscle, only unbound metabolites present in millimolar concentrations give rise to distinct peaks. Five major peaks are seen in muscle and these include three from the ATP molecule, one from phosphocreatine (PCr), and one from inorganic phosphates (Pi). MRS can provide diagnostic information on the different cellular levels of high-energy phosphates.

Appendix 2: Exercise Tests

1. Forearm Non-ischaemic Exercise Test

Please note that since the ready availability of molecular genetic testing, and due to issues of painful contractures and many false positive/negative results, this test has been rendered almost obsolete.

Purpose

Test for muscle phosphorylase deficiency (McArdles disease) or other glycolytic defects, and myoadenylate deaminase deficiency.

Method

Fast patient from midnight and avoid exercise for 30 minutes before test starts. Insert an arterial cannula into the antecubital vein then rest the patient for a further 10 minutes. Keep the line patent with heparin.

Take a baseline resting sample for lactate, ammonia, urate, phosphate, carnitine, CK and renal profile and any other tests required for general investigation.

Exercise the forearm by squeezing a sphygmomanometer bulb to exhaustion (usually for 1-2 minutes). Pain may be experienced which is normal; despite this determined exercise should be encouraged to avoid equivocal results.

At the end of exercise sample for lactate and ammonia at 1, 2, 4, 6, 8 and 10 minutes.
Each sample must be processed immediately.

Also at end of exercise clinically examine the forearm for contracture and the fingers for flexure. A rigid forearm or inability to extend the fingers may be an indication of metabolic muscle disease.

Interpretation of Results

Normal response is a significant rise, usually at least 3-5 fold, in lactate and ammonia. No increase in lactate indicates a defect in the glycolytic pathway. No increase in ammonia suggests myoadenylate deaminase deficiency. A generally poor response in all parameters may reflect inadequate exercise.[3]

2. Aerobic Exercise Test

A 12 minute walking test is now seen as the gold standard to quantify aerobic fitness in GSDV.

Other timed walk tests (usually 6 minutes) are used for a similar purpose in other conditions such as Pompe.

A cardio pulmonary exercise test (CPET) can also be completed on a bike or treadmill. (information from personal communication with Andrew Oldham, Metabolic Physiotherapist, Salford Royal Foundation Trust).

Purpose

For the investigation of aerobic exercise capacity in patients with possible mitochondrial respiratory chain disease and to observe the 'second wind' phenomenon in McArdle's (GSDV).

Patient

Samples
2 ml EDTA blood for ammonia.
5 ml heparinised blood for urate, phosphate, potassium and other tests that may be required.
Blood collected into a fluoride tube for lactate.

Method

Stress patient with bicycle ergometer. The level of exercise is set to achieve an output of 50 watts.

Exercise for 15 minutes taking samples at 0, 5, 10 and 15 minutes
With the patient at rest take further samples at 30, 40 and 60 minutes.

Interpretation of Results
Provided the patient does not become anoxic there should be little, or no, change in metabolite levels.

Appendix 3: Specialised Laboratory Investigations (muscle biopsy - histology, enzyme analysis and mutation analysis on blood)

Specialised Lab Investigations	
Muscle biopsy **Histology** Evaluation of muscle fibres Staining for lipid amylopectin and glycogen Immunohistocytochemistry Electron microscopy of muscle **Enzymology** Specific enzyme assays: - Enzymes of glycogen metabolism Enzymes of glycolysis Respiratory chain enzymes	**Skin biopsy** **Enzymology** Fatty acid oxidation flux CPT2 specific assay **EDTA blood Mutation analysis** (Common mutations) AMPD1 c.34C>T (p.Q12X) PYGM c.158C>T (p.R50X) in exon 1 PYGM c.613G>A (p.G205S) in exon 5 CPT2 c.338C>T (p.S113L) in exon 3

Muscle Biopsy

When taking a muscle biopsy several factors need to be considered as these may impact on the biochemical findings (e.g. anaesthetic influence, structural defects in muscle, lack of standardised handling and processing) and other additional environmental influences to the patient (e.g. genetic background, existing therapies at the time of biopsy, environmental triggers).

For quantitative biochemical studies, it is important to take the biopsy from a clinically-affected muscle but not from an area that represents end stage disease. Most teams would biopsy either the quadriceps or tibialis anterior. Furthermore, if a local anaesthetic is used, it is important to avoid taking the biopsy from the site where the anaesthetic is administered as this may affect the result. It is important to note if the patient is on any co-factor / vitamin / carnitine therapy or IV glucose at the time of the biopsy as these therapies can up-regulate respiratory chain function or lead to high levels of stored muscle glycogen. Correct orientation of muscle fibres is vital for full evaluation. The muscle specimen should be placed in a dry pot (for example a deep 22mm diameter petri dish with lid) which is then put in the fridge if not handled immediately. A specimen in a dry pot in the fridge (or on ice) is good for up to 2 hours for histochemistry and most "fresh" biochemistry but is not so good for EM. An aliquot of the biopsy should be snap frozen at the bedside in liquid nitrogen and stored at – 80 ^{O}C for homogenate studies i.e. respiratory chain complexes and enzyme studies. An aliquot of tissue should also be processed for paraffin histology and electron microscopy. If molecular studies are pursued, tissue from any of the above can be used for

DNA analysis. Typically, 80-100 mg tissue is required for the assessment of respiratory chain enzyme activities.

The laboratory analysing muscle biopsies **MUST** be contacted prior to undertaking a biopsy and they will supply their local information. **NB** Muscle which has been in contact with OCT (histochemistry mounting medium) is NOT suitable for assay of glycogen or glycolysis enzymes. OCT is a complex polysaccharide and interferes with these assays. Experience suggests that using saline soaked gauze to keep a muscle biopsy fresh in a petri dish renders the biopsy fairly useless; washing out some of the enzymes; causing ice crystal and EM artefacts.

Muscle Biopsy Analysis	
BIOCHEMISTRY	**HISTOLOGY**
FROZEN (homogenate) Glycogen quantitation Glycogen structure RES complexes I, II, III, IV*, ubiquinone GSD enzymes e.g. Types V, type VII, PbK Glycolytic enzymes e.g. PGK, PGM, LDH FRESH (intact mitochondria) RES complexes I, II/III, IV Polarography – oxygen consumption with various substrates / inhibitors ATP synthesis (complex V) SDS electrophoresis / Western blotting for membrane associated abnormalities	Abnormal enzyme reactivity (SDH, COX, COX+SDH, NADH, PPL, PFK, AMPD) Abnormal storage – lipid, glycogen Abnormal Gomori trichrome staining – RRF Also ragged blue fibres on SDH staining Abnormal fibre architecture e.g. myopathy / dystrophy **Electron microscopy** Abnormal mitochondrial structure and / or numbers Glycogen pooling, lipid accumulation

*UCLH measure I, II + III and IV. Newcastle measure I, II, III and IV (i.e. all enzymes individually)

AMPD – myoadenylate deaminase, PPL – (myo) phosphorylase – McArdle's disease (GSD type V), PFK – muscle phosphofructokinase (GSD type VII), PbK phosphorylase b kinase, PGK phosphoglycerate kinase, PGM phosphoglycerate mutase, LDH lactate dehydrogenase, RES – mitochondrial respiratory chain enzymes, GSD – glycogen storage disease, SDS sodium dodecylsulphate polyacrylamide gel electrophoresis, SDH - succinate dehydrogenase, COX – cytochrome oxidase (complex IV)

Enzyme analysis on cultured fibroblasts

A skin biopsy is recommended for the investigation of fatty acid oxidation defects as enzyme analysis in muscle is generally much less reliable. Flux assays in fibroblasts using [9,10-^{3}H]myristate, [9,10-^{3}H]palmitate and [9,10-^{3}H]oleate have the potential to pick up "mild" VLCAD, CPT2 deficiency and mild mitochondrial trifunctional protein deficiency (TFP). Mild TFP deficiency can present with isolated peripheral neuropathy and episodic rhabdomyolysis in both children and adults. However, CPT2 activity should be assessed by specific enzyme assay, particularly as partial deficiencies of this enzyme can lead to clinical disease but may not be detected by flux assay.

The mitochondrial diagnostic laboratory at Newcastle also offer respiratory chain enzyme measurements in fibroblasts within its accredited scope.

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Definitions

- CK = creatine kinase
- Myocardial infarction = detection of increased cardiac high sensitivity troponin values above the 99th percentile reference limit and occurrence of the rise/fall of cardiac troponin values [1]
- EMG = electromyography (measures muscle response or electrical activity in response to a nerve's stimulation of the muscle)
- ECG = electrocardiography (recording the electrical signal from the heart to check for different heart conditions)
- ^{31}P MRS = ^{31}P magnetic resonance spectroscopy (measures phosphorus containing metabolites such as phosphocreatine in the brain and skeletal muscle)

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